

nature*1 September 1977*

South Africa's nuclear intentions

WORLDWIDE alarm over reports that South Africa is on the verge of joining the nuclear weapons club were temporarily stilled last week when Prime Minister John Vorster privately assured the United States that South Africa is not developing nuclear weapons. The assurance is welcome, indeed, but it would be even more comforting if South Africa were to back up its protestations of innocence by signing the nuclear non-proliferation treaty and by placing its nuclear facilities under international inspection, steps which Mr Vorster's government has consistently refuse to take. The flap over South Africa's intentions does, however, have some positive aspects.

The alarm was first raised on 9 August by the Soviet press agency, Tass, which warned that South Africa was about to explode a nuclear device. A few days later, Tass charged more specifically that plutonium for the country's nuclear weapons would come from two 1,000MW reactors that France recently agreed to supply to South Africa. Since the reactors will not be in operation until 1982, and the agreement requires spent fuel from them to be reprocessed in France with the extracted plutonium remaining in French hands, the Soviet charges were hard to believe. Nevertheless, they prompted the United States, Britain, France and West Germany to take the matter up with Mr Vorster and his government.

The public debate was fuelled further last week when Louis de Guiringaud, France's foreign minister, announced in an interview that France also has new information indicating that South Africa was preparing to test a nuclear device. Dr Guiringaud warned that such a test would have "grave consequences", which would presumably include shelving the sale of the two French reactors and further political and economic reprisals.

Although few details of the basis for de Guiringaud's announcement have been revealed, it is widely believed that the concern was based on evidence of extensive construction work in the Kalahari Desert. The evidence was reportedly obtained by satellite and may have sparked the original Tass pronouncement.

South Africa's response to all of these charges was initially to protest that its nuclear activities are entirely peaceful, a protestation that left room for doubt since it

should be recalled that India's explosion of a nuclear device in 1974 was also labelled as peaceful. At his press conference on 22 August, however, President Carter announced that the United States has been given a more complete assurance that South Africa is not intending to develop nuclear weapons.

Specifically, Carter announced that "in response to our own direct inquiry and that of other nations, South Africa has informed us that they do not have and do not intend to develop nuclear explosive devices for any purpose, either peaceful or as a weapon—that the Kalahari test site, which has been in question, is not designed to test nuclear explosives, and that no nuclear explosive tests will be taken in South Africa, now or in the future". Carter added, however, that the United States would "continue to monitor the situation very closely".

Some comfort should be taken from the fact that as soon as allegations were first raised about South Africa's nuclear intentions, four major western powers moved swiftly to put South Africa on notice that a nuclear explosion would have grave political consequences, and that South Africa would not be allowed to hide behind the pretence that an explosion could be called peaceful. South Africa's unequivocal denial of plans to develop nuclear explosives is a welcome statement.

Nevertheless, until South Africa agrees to submit its nuclear facilities to international inspection, suspicions will linger. It should be noted, for example, that the country has large reserves of uranium and a pilot-scale enrichment plant, perhaps capable of enriching uranium to weapons grade. Experts in the United States have publicly stated that they believe South Africa has the technical ability to produce explosives, given sufficient concentration of funds and manpower on the effort. South Africa has now been put firmly on notice that any such attempt would lead to grave political consequences including possible abrogation of trade and economic agreements, but Carter also noted last week that the United States would "renew our efforts to encourage South Africa to place all their nuclear power production capabilities under international safeguards and inspections". That should be a major political priority. □



Technik: the relevance of a missing concept

M. Fores, of the UK Department of Industry, and I. Rey, previously of the Swedish Ministry of Industry, offer their personal suggestion that the absence of an English word to describe the 'useful arts' of manufacture leads to unnecessary confusion in talking about the world of manufacturing

WRITERS in the English language often use words in such a way that 'science' and 'technology' are considered as key features affecting manufacturing. Sometimes they are even wrapped together and simply known as 'science'. Hilary and Steven Rose, for instance, do this in their book *Science and Society*. Such a usage incorporates a number of errors concerning what was once known as the 'useful arts' of manufacture. To show this, we introduce the word *Technik* which is widely used and understood in Germany. It concerns the functioning of natural and man-made things, including the set of principles according to which artifacts work and the methods used in making them. Most continental European languages include such an idea.

One English language study which erred over the general influence of 'science' in the culture was Bronowski and Mazlish's *The Western Intellectual Tradition* (Penguin). They wrote:

What is needed is that in the general history books the development of science should take its place along with political and economic developments. The steam engine helped to shape the modern world at least as much as Napoleon and Adam Smith, but only rarely do historians admit the fact . . .

Few would dispute that the steam engine has had an important influence on the modern world; but it cannot sensibly be thought of as a 'product of science'.

In Germany, if there are two cultures or sub-areas of the general culture, they are not the science and humanities of the English split. They are *Wissenschaft*, concerned with all knowledge and all the subjects of the classical university, and *Technik*. Normally, however, there are three sub-cultures: *Kunst* 'art', the English 'fine arts' and performing arts; *Wissenschaft*; and *Technik*, widely accepted to be separate from both. *Wissenschaft* means the art of handling knowledge, akin to 'gamesmanship' and seamanship'.

The importance of *Technik* stems from the fact that it has been a prime concern of the species from earliest times. Some of its first products were stones sharpened to kill animals for food, or to ward off dangerous animals. Man became a tool-maker and tool-user through *Technik*.

The history of *Technik* is not, however, the conquest of nature. Problems of *Technik* are not seen in terms of nature but rather of a specific job to be done or an artifact to be made. And more complex contrivances strayed further from the natural state of their component materials. A stick, as a lever, was made of wood, directly from the natural world. But a potter's wheel could be made from various materials, probably in combination. And a governor to control the speed of a rotating machine is a contrivance to control a contrivance. Thus the exponent of *Technik* must be obsessed with the artificial, rather than the natural. If the operations of *Technik* shade into those of either of the other two cultural areas, they shade into *Kunst*, rather than *Wissenschaft*. Metal-working skills are an obvious example of craft skills that have been shared between making the products of fine art and useful artifacts.

The 'applied science' concept is a peg on which some have hung their views about manufacturing. Only in English, of the major European languages, can the concept exist, since only English has a meaning for the word science which only includes knowledge of natural pheno-

mena, or the process by which this knowledge is derived. By use of the 'applied science' phrase, the process of *Technik* is made to seem closer to the natural world than it is in practice.

The applied science concept is misleading for other reasons. It implies that a contrast can exist with pure science. Yet there can never be any body of pure science at all, if the criterion used is lack of utility, or lack of applicability of knowledge. All scientific knowledge is put out freely for verification of falsification—so all can be used freely and with no attribution. The most basic ideas of natural science, such as Newton's laws of motion, turn out to be widely applicable in practice. So the bizarre conclusion must be drawn that the purest of science is more widely applicable in practice than work which is done for particular uses, so-called applied work. Applied science as a sensible near-alternative to *Technik* fails because manufacturing cannot be well characterised as the application of scientific knowledge. Furthermore, studies have shown that the rate of technical progress in manufacturing cannot be equated with the rate of adoption of science.

Continental-style *technologie* invariably means what one would expect from its Greek roots (*techne* means art; *logos* means word or law), so it is not the same as English-language technology. British official statistics show that most technologists are engineers; they are not like other types of '-ologists', students of events. The use of the concept of *Technik* avoids this misunderstanding.

Another difference between perceptions of *Technik* and of technology comes with the idea of utility. Acts of *Technik* are accepted to be pointless if there is no demand, within the existing cost pattern, for the useful artifact being made. There are no imperatives of *Technik* along the lines of technological imperatives of the English-language, whereby critics complain that because something can be made it will be made.

Another feature is that *Technik* does not rule well in an aristocratic or a dictatorial situation, if only because it depends more on detail than on principle. The best conception for a contrivance, or the best design for it, is of no use if the detailed manufacture of component parts is not up to standard. The whole may not be greater than the sum of the parts; but if one of the parts is defective, the whole cannot function.

Continental *Technik* is accepted to be a separate cultural area with codes of behaviour, responses and a dignity of its own. The competent *Techniker* does not feel that he has constantly to look over his shoulder at science or the arts, as the exponent of technology often does. Science and arts do not care much for utility and they have a different grasp of the artificial. *Technik* remains a missing concept in English; its absence is the English speaker's loss. □

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Mary Evans

Potter's wheel : an example of Technik

The French 'nuclear war'

Les écologistes are gaining a voice in French politics. Alexandre Dorozynski outlines their growth

THE French government is currently faced with a political issue it would doubtless have preferred to keep out of the public forum: that of its "all out" nuclear power development programme and, more particularly, of the fast breeder Super-Phénix reactor at Malville, near Lyon. The issue has become political as the campaign preceding the nationwide legislative elections to be held in March next year has got underway. Polls indicate that the election may well give the Socialist-Communist coalition an edge in the National Assembly, and it now seems likely that the 'nuclear weapon' will be used in the forthcoming 'battle'.

Socialist leader François Mitterand has already suggested that a national referendum be held on the nuclear programme. And a loosely-knit group, termed somewhat vaguely and sometimes disparagingly as *les écologistes*, are beginning to represent a political power to be counted with. Under a programme termed *Écologie '78*, they are to be represented at the municipal elections next year. Seven ecological groups will put forward candidates.

Les écologistes first emerged as a political group in 1974 under the leadership of agronomist René Dumont, who received 1.34% of the votes cast during the presidential election that year. Since then, their numbers have increased rapidly: during the municipal elections earlier this year, they mustered an unexpected 10% of the votes. So far, they have refused to support any of the existing political parties, but if they decide to throw their weight one way or the other, they could well alter the balance between right and left.

But who are *les écologistes*? According to the Ministry of the Environment, created in 1971, there are nearly 15,000 ecological associations in France. They

include such groups as the National Federation for the Protection of Nature, created in 1968, now with a membership of about 100,000; the Association for the Rights of Pedestrians (15,000); the French Association for the Protection of Water (about 1,000); and smaller, regional and special interest groups such as the Friends of the Trees of the Loire (500) and the Committee against Atomic Pollution of La Hague (150). Some of these associations overlap, and total membership is probably no more than two million. The first associations were started with specific, sometimes narrow goals, but interest gradually grew to include more general issues concerning modern life; consumerism, means of production, housing, and so on.

Nuclear power concern

One of the major issues now concerning *les écologistes* is the nuclear power programme and the construction of Super-Phénix, which was the bone of contention of some 50,000 marchers who converged on the farming region of Malville, 40 miles from Lyon and 30 miles from Geneva on Sunday 31 July. They clashed with a few thousand police and a paratrooper unit in what has been called the first battle of the French 'nuclear war'. The battle had its casualties—about 100 wounded and one dead. The dead man, Vital Michalon, was a quiet, young teacher of physics and chemistry (and a reserve officer) whose death has been variously attributed to heart failure, to the explosion of a concussion grenade used by police, or to that of an amateurish explosive device put together by the less peaceful *manifestants*.

The 'march on Malville' might have been dismissed as the kind of incident likely to occur during a hot summer (even though the summer was cool and the marchers were soaked by rain) but public polls, in which Frenchmen participate eagerly, indicate a mounting popular concern which the government cannot dismiss. In 1974, three-quarters of representative samples of Frenchmen were "very much in favour of or rather in favour of" the development of nuclear energy. At the beginning of this year, the percentage in favour had fallen to 50%, while that against had risen from 17% to 41%.

A poll carried out for the weekly news magazine *Le Nouvel Observateur* after the 'march on Malville' showed up the somewhat conflicting views of the French on the nuclear issue. Although nearly 70% considered an

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Malville: the first battle

increase in energy consumption to be indispensable to the improvement of their way of life, nearly 50% would be "willing to change their consumption habits and way of life if this could stop the construction of nuclear power plants". A majority (56%) approved of Mitterand's suggestion for a referendum on nuclear power. Of those questioned who identified themselves with the Communist party, 76% approved of the proposed referendum, which Georges Marchais, the Communist leader, has since vigorously condemned.

It now seems impossible to keep the public debate from spreading, and politicians, right and left, may be called upon to take sides. The Ministry of the Environment is bound to go along with the government's pro-nuclear stand, especially as Michel d'Ornano (he is the one who lost the Paris mayoralty to Jacques Chirac), now Minister for Culture and the Environment, used to be, as Minister for Industry, one of the proponents of the "all nuclear" power plan. And the importance of the forthcoming debate has grown in the light of recent talk of a moratorium on the construction of new nuclear power plant in Germany.

So what do the ecologists really want? Few of them will deny that, in view of the poverty of French resources in oil and coal, and the built-in limitations to the development of hydro-electric power, some of France's energy should be nuclear. But most consider the intensive programme unreasonable, and the development of Super-Phénix risky. The government, on the other hand, views nuclear power as a means of stopping the outward flow of currency, and the fast breeder as a major trump to play in case of uranium scarcity in the future (and, also, as a potentially marketable technology).



Michel d'Ornano, environment minister

Popperfoto

French embassy

It is certain that without intensive development of nuclear power, the 'official forecast' of 370×10^9 kWh of electricity by 1985 will not be reached, by far. But is this a forecast or a policy statement? Less than half of 370×10^9 kWh of electricity was produced in 1975, and less than one-tenth of that figure in 1950. Ecologists do not fail to remark that Eurodif, Europe's first uranium enrichment plant, at Tricastin, is itself expected to consume some 30×10^9 kWh a year, which is almost as much as the total electrical energy output in 1950. And they also argue that a less spectacular increase in energy production may not mean, as hawks have hinted, a return to the Middle Ages.

Marching for other issues

But nuclear power is not the 'ecologists' only concern. On 15 August, protesters marched on the Larzac plateau, in the southern part of the Massif Central, against the Army's proposed take-over of more than 10,000 ha of land for training and testing. The expansion was planned in 1970 and would have been completed last year, were it not for the deter-

mined opposition of local farmers, who have succeeded in mustering considerable support. In six years of stubborn but largely peaceful opposition, the farmers have become well organised and have learned many tricks. For example, they published a study of cattle and sheep farming in conjunction with an effective public relations operation which included releasing a flock of sheep under the Eiffel tower in Paris; on another occasion they organised a symbolic harvest for the Third World which opened a debate on malnutrition.

The success of the Larzac farmers, who have joined the loose ranks of *les écologistes*, is measured by the fact that the Army has not been able to acquire half of the land it wanted, and that sheep are still squatting on some of the Army-owned land in virtue of ancient pasture rights. Conscious of public opinion, the Army has seldom resorted to expulsion by force.

Les écologistes have also contributed to the sinking of a proposal to construct a super-highway in Paris (the *Radiale Vercingétorix*, named after the famous Gaul warrior), to preserving some sites from urbanisation, to

promoting legislation against water pollution, to imposing controls on the asbestos industry, and to banning the 2-4-5T defoliant. But it should not be forgotten that they are a variegated group, which includes the scientifically inclined, philosophers, and dreamers who yearn for the unpolluted paths cherished by Jean Jacques Rousseau and probably gone forever.

Many of them, and the public, simply do not recognise the boundary between dream and reality because they are not well enough informed. Most, however, appear to have a genuine concern about the environment as a part of society that has changed irreversibly, and believe, as voiced by Giscard d'Estaing at a ministerial council last July, in "the importance of environmental policy as a factor of transformation of society".

As Frenchmen shift to high gear in their semi-permanent political campaigning, the hope is for a reasonable, informative debate. Perhaps then the traditionally central authority, and the inevitably peripheral, but increasingly concerned, citizen will find a common point. □

USA

New DNA draft

An important new factor has been injected into the long-tangled process of developing legislation to control recombinant DNA research in the United States. Colin Norman reports

WHEN members of Congress left Washington for their summer vacations a month ago, two separate versions of legislation to control recombinant DNA experiments were under consideration. In the Senate, a bill developed by Senator Edward Kennedy's Health Subcommittee was awaiting consideration by the full Senate, while in the House, a bill drafted by Representative Paul Roger's Health Subcommittee was pending before the House Commerce Committee. When Congress returns next week, however, a third version will have to be taken into serious consideration, and the already murky legislative outlook has become even more opaque. In fact, there is now a real possibility that Congress will not complete its work on the legislation this year.

The new version has been drafted by Senator Gaylord Nelson, a Democrat from Wisconsin, in response to concerns which a number of scientists have voiced over the Kennedy bill. Nelson

says he plans to offer his bill as an alternative to Kennedy's when the legislation is brought to a vote in the Senate, and the move will guarantee a full-scale debate on some of the key issues.

The Kennedy bill has aroused some concern because it would establish a Presidentially-appointed commission to draft new rules for recombinant DNA experiments, licence facilities and approved experiments, an arrangement which many scientists view as a recipe for bureaucratic delay. The bill would also establish hefty fines for those who violate the rules and it would allow state and local governments to write regulations which would be stricter than the federal controls. The House version would be less restrictive since it would leave most of the responsibility for implementing controls in the hands of local biohazards committees, and it would also place considerable restraint on the authority of local governments to set their own recombinant DNA regulations.

Until recently, the Kennedy bill was moving through the Senate without encountering much Senatorial interest or opposition, and it was scheduled for a vote in the full Senate shortly before the August recess. It was shunted aside by more pressing legislation, however, and it is still pending. The delay has

enabled a number of scientists to step up their lobbying, and their concerns have met with a sympathetic reception in Nelson's office. Nelson's proposed bill is very similar to the House version, though in some respects it is even less restrictive.

Though Nelson has not actively sought supporters for his bill, his office has been receiving a number of calls from other Senate offices expressing interest. In addition, other Senators, including Moynihan of New York, Eagleton of Missouri and Chaffee of Rhode Island, have voiced concern about Kennedy's proposal to establish a new recombinant DNA commission. In short, Nelson's bill is likely to gather considerable support. Nevertheless, if it is put to a vote and it goes down to a heavy defeat, those who support the Kennedy bill would have their hand considerably strengthened when negotiations begin to iron out differences between the House and Senate bills.

Meanwhile, the Roger's bill is not likely to be approved by the Commerce Committee much before the end of September, and it may then be referred to the House Science and Technology Committee. That would delay passage by the House until mid-October at the earliest, which means that there would be little time to reach a compromise with the Senate version before Congress goes into recess before the end of October. □

IN BRIEF

EEC research

The European Commission has recently sent proposals for a new European science and technology policy to the Council of Ministers. In a communication called *The Common Policy in the Field of Science and Technology*, it suggests a new structure for cooperation and some areas where collaborative research should be increased.

Since 1973, energy has taken 64% of the Community R&D budget. The proposals say that this balance should be redressed and suggest that a new programme of forecasting and assessment in science and technology (FAST) be set up. FAST will be a collaborative effort between research groups in Europe and international bodies with an initial budget of 4.4 million ua (1ua=£0.66).

ICRF sues for libel

Lawyers for the UK Imperial Cancer Research Fund (ICRF) have recently issued writs for libel against Daily Mirror Newspapers Ltd. The legal action has arisen over an article published in the *Sunday Mirror* on 14 August, questioning whether some of the £23 million which the ICRF has invested could be put to better use.

The ICRF replied to the article in a statement issued on 15 August which explained that such a high level of investment was necessary to support long term research. The decision to sue for libel was taken after the *Sunday Mirror* refused to publish an apology.

Engineering inquiry

Following hard on the heels of the UK

Department of Education and Science's (DES) discussion paper *Industry, Education and Management is Education, Engineers and Manufacturing Industry*, a report prepared for the British Association for the Advancement of Science (BAAS) under the direction of Dr Joseph Pope, Vice Chancellor of Aston University. Like the DES publication, the BAAS report is the outcome of an investigation into ways of encouraging able professional engineers to enter British industry.

The report puts some of the blame for industry's ills on the low status of engineers, which discourages young people from entering the profession. It recommends that universities and polytechnics accept candidates without 'A' level physics. More women should also enter engineering.

THE principal mountains of California, the Sierra Nevada, run in a north-south chain. They are unbroken by highway crossings for 165 miles of their greatest height. Within this vast area, about 40 miles wide by trails, are two National Parks, Kings Canyon and Sequoia. North of Kings Canyon Park is the equally splendid Sierra National Forest. The flank of the Sierra lying east of the summit crest is outside the park boundaries, and within Inyo National Forest. Much of the terrain outside the parks has been designated as Wilderness Area, administered by the US Department of Agriculture. Happily, there is collaboration between the Departments of the Interior and Agriculture in administering this scenic treasure, which in recent summers has swarmed with knapsackers. In 1976, for example, it received 1.1 million visitor-days of use.

"The Earth was created by the assistance of the Sun, and should be left as it was". So, we are told, said Chief Joseph of the Nez Percé. Much of the Earth is no longer as it was, but Chief Joseph's concept is still the guiding principle for managing wilderness. Detailed instructions of the observation of this principle are given to human invaders at the entry points to the wilderness, which are monitored. Yet there are anomalies. One of them is the presence of saddle horses and pack mules, whose excretory reflexes seem to be stimulated while wading through streams. These animals, however, are allowed to mar the wilderness; they have a strong hold on western American traditions.

Another interesting problem is that pet dogs are permitted in wilderness

areas that lie outside the boundaries of national parks. I have just returned from the John Muir Wilderness Area and its visiting dogs. One of the most prominent breeds was the Labrador retriever, a dog that

Wilderness areas



THOMAS H. JUKES

enjoys jumping in a mountain lake to fetch a piece of wood. The name of the canny Scotsman, Muir, has been given to many other California landmarks, including schools, a hospital, a national monument and a famous trail. Perhaps Muir's wilderness area should be re-named for his dog, 'Stickeen'. An even better idea would be to exclude the dogs, so that native mammals would be less harassed.

A further anomaly: hunting, in season, is permitted in wilderness areas of national forests, but not in national parks. Sharpshooters lie in wait just outside the park boundaries

to pick off deer that heedlessly step outside the demilitarised zone. The official instructions issued to wilderness users remind them, almost plaintively, that target practice with firearms is illegal, but hunting is not.

Our camp was evidently in the territory of a marmot. These beasts usually greet intruding hikers with a shrill whistle, and then scurry into holes. This one walked into camp, apparently to be photographed, after which it fixed us with a withering stare, and refused illegal offerings of food. Another frequent visitor to camps is a raucous bird, Clark's nutcracker. Now I know that the regulations say that the entrails of fish should be carefully buried at least 100 feet from water, but Clark's nutcracker would much rather devour them on the spot, and I could not resist his supplication. Next morning, at daybreak, he awakened me in the whitebark pine just above my head with a loud scream to demand breakfast.

At timberline, about 11,000 feet, the flowers make a mockery of all amateur and professional gardeners. Last week, in Pioneer Basin, it was the time for blue, mauve and pink penstemons: small blue-and-white lupines, golden bush potentillas; rose epilobium, and both large and tiny yellow monkey flowers. Eastern brook trout (*Salvelinus fontinalis*) in the high lakes scorned, for the most part, the offerings of anglers. There were some splendid afternoon thunderstorms, the nights were clear and star-filled, and the mornings blazed with sunlight. In general, the visitors, with their small tents and bright clothing, were heedful of Chief Joseph.

correspondence

Sinking of 'Cavtat'

SIR,—With regard to the report by Alastair Hay (26 May, page 301), on the sinking of the Yugoslav ship 'Cavtat', off the coast of Otranto in southern Italy, I understand that there is no evidence that poisonous lead derivatives have leaked from the drums in which they are sealed.

The article, however, is decorated with the picture of an Italian fisherman holding fish, with the caption "Poisoned fish caught in the Gulf of Taranto". That picture and caption will certainly capture the attention of most readers of *Nature*, and it will mislead them into thinking that if they visit that area, they will be at high risk of being poisoned. Because this is clearly not the case, the above picture seems ungenerous toward the people of the area, who are heavily dependent on tourism and may suffer from the effect of an unfortunate picture and caption published in an authoritative journal like *Nature*.

FRANCESCO BRESCIANI

Department of Pathology,
University of Toronto, Canada

Effects of marine research

SIR,—Each time I re-read Desmond Scott's assessment (30 June, page 762) of the effects on marine research of the Third United Nations Conference on the Law of the Sea, I get more annoyed. It is not that his forecast seems improbable—coastal states are indeed likely to exert total control over all scientific research on their continental shelves and within 200 miles of their shores. But the account of how this situation arose and how it should be cured exhibits a curious bias.

Scott appears to attribute all political difficulties between developing and developed countries, especially as they pertain to ocean resources and jurisdiction, to the marine scientists. His view of them is surprisingly bitter and unfortunately misleading. I contend that the scientists had little to do with creating the problem and can do little to solve it. In the confrontation between the developed and developing countries, related as it is to the history of colonialism and its children, post- and neo-colonialism, and accompanied by the emergence of the 'new economic order', marine science has played a pretty trivial role.

The issue of control over marine

science was initially evoked for bargaining purposes, could well have been dropped once the question of jurisdiction over coastal resources was settled, and now refuses to go away, to the great eventual cost of all who use the ocean and its resources or are affected thereby. International interactions under the new law of the sea will be among governments, not individuals, and there are few governments whose actions are determined by scientists, especially when larger political forces are at play.

WARREN S. WOOSTER

Institute of Marine Studies,
University of Washington, USA

Harvesting whales

SIR,—In your 'in brief' columns (16 June, page 576) there is a confused and inaccurate account of some analyses I have done on the dynamics of harvested populations. Particularly misleading is a belief attributed to me that certain whale species are under the threat of extinction due to the dynamic instability of the harvesting strategy. I have never made any statement to this effect and do not believe it to be true. Indeed the idea is absurd.

JOHN BEDDINGTON

Department of Biology,
University of York, UK

Darwin and his flowers

SIR,—In view of your reviewer's acceptance of "the role of plants in Darwin's scientific life," (21 July, page 273) it may be of interest to mention that in a collection of unpublished letters from eminent botanists and gardeners, written to the late William Robinson, editor of *The Garden* (1871–1927), and preserved by a member of this Association related to Robinson, are some written by Charles Darwin from Downe, Kent in 1875.

On 4 January he wrote: "I am much obliged for your kind gift of six copies of *The Garden*, with a drawing of myself and a notice of my works. This notice is drawn up in the most generous spirit and is highly honourable to me. I thank the writer very sincerely."

In a further letter he wrote: "I may mention (though it is improbable) that you can aid me on one point. If you have some *Eurhale ferox*, and if it produces more than one flower at a time, I wish you would cross some and fertilise some others with their

own pollen, in order to see, when their seeds are counted, whether the cross aids at all in increasing their fertility. The *Eurhale* is dead at Kew where they would have made the trial on a large scale for me. Professor Carpavy (?) has advanced this plant as a case of self-fertilisation for many generations with unimpaired fertility."

He also asked for some experiments with two distinct plants of *Nymphaea* which were protected while expanded from insects. On 5 May he wrote: "I write . . . to thank you and to say that it will be superfluous to castrate the flowers which are crossed with a pollen from a distinct plant, grown under as different circumstances . . . as maybe. . . . The flowers fertilised with their actually own pollen should certainly be protected from insects. . . . I was glad to see you elected to the Linnaean Society."

Charles Darwin's chief contribution seems to have been to make the world realise that plants have a history as well as a structure which had been the sole concern of most botanists.

ERIC HARDY

Merseyside Naturalists' Association,
Liverpool, UK

Grants for social science

SIR,—The article 'Research on research' (4 August, page 398) refers to the sum of £25,000 transferred to the Social Science Research Council (SSRC) from the Council of Scientific Policy (CSP) for science policy studies. You state that the research grant to the Science Policy Research Unit (SPRU) at the University of Sussex "means that other applications are denied". This is untrue, and is a misunderstanding of SSRC's procedures.

The grant made to SPRU is under the SSRC's research grant scheme, and the funds come from the general research grant allocation. The sum transferred to SSRC from CSP for science policy studies is at the disposal of the Research Initiatives Board (RIB) for research contracts. Should the RIB and Council decide to give greater priority to science policy studies it is up to them to take a further initiative in this area.

It is therefore untrue that the research grant to SPRU closes the door to other applicants.

DAVID WAINWRIGHT

Social Science Research Council,
London, UK

news and views

Nucleosome core structure

from Michael Spencer

THIS issue of *Nature* (page 29) contains the first results of an examination of crystals made from chromatin core particles. The analysis, by Finch *et al.* of the MRC's Cambridge laboratory, is an impressive result of applying all the resources of a multidisciplinary group to rather limited data, so as to extract from it the maximum amount of information.

The core particle is a repeating unit containing 140 base pairs of DNA and an octamer of basic proteins, and is a degradation product of the complete nucleosome which (in rat liver) contains 200 base pairs and an extra protein. Earlier workers on nucleosomes have used electron microscopy, X-ray and neutron diffraction, and biochemical techniques, to build up a tentative picture of a somewhat asymmetrical particle with a hole in the middle, the DNA being wrapped round the outside. A model which anticipated some of the features seen by Finch *et al.* has been described by Richards *et al.* (*Cell Biology, Int. Rep.* 1, 107; 1977). Finch *et al.* have now established a model of this type in a rigorous manner and filled in some new details, by developing a large scale preparation method for homogeneous core particles and obtaining single crystals.

Crystals were first obtained in 1975 by Bakayev *et al.* (*Nucleic Acids Res.* 2, 1401), but they could only record powder diffraction patterns because the crystals were too small. Even now the analysis is restricted to details larger than about 20 Å, either because of the disorder within the crystals or because there is a combination of small crystal size and large unit cell. It is enough, however, to define a probable path for the DNA and to suggest some features of the protein core. An interesting new technique, which will surely be applied

to other large structures in the future, has been the use of electron microscopy to deduce the phases of the X-ray reflections. This vital information, without which a structure cannot be solved, is often deducible only with the aid of isomorphous derivatives which in the present case were not available.

Finch *et al.* got round this difficulty by taking electron micrographs of small fragments of single crystals, and obtaining optical diffraction patterns from the micrographs. Some of these patterns could be identified with the three different X-ray patterns obtained with each of the unit cell faces at right angles to the beam (the 'principal projections'). Having done this, they calculated Fourier transforms from the density distributions in the micrographs and used these to deduce phases (or signs) for most of the X-ray amplitudes. The remaining signs, which were needed to extract the fine detail information present in the X-ray patterns, were deduced by trying different combinations of signs until the three independent particles in the unit cell looked as similar as possible in the calculated electron distribution.

The picture that emerges is of a roughly disk-shaped particle of diameter 110 Å and thickness 57 Å, divided into two symmetrically arranged halves along its short axis and somewhat wedged-shaped, like a half-open oyster. The authors have, with Cantabrigian erudition, coined the name 'platysome' to describe it. The DNA cannot be positively located, but the electron distribution suggests that it forms a superhelix of pitch 28 Å round the outside of the protein core. Now enzyme digestion studies by a number of workers (for example Simpson & Whitlock *Cell* 9, 347; 1976) show that nucleosome DNA chains are most sensitive to attack at intervals of 10 bases along their length. This is consistent with a DNA double helix of 10 base pairs per turn wrapped round a protein core. There are, furthermore,

tracts of relatively low sensitivity which suggest that the protein is in closer contact with DNA at two points in each turn of the superhelix. From this Finch *et al.* deduce that the DNA occupies $1\frac{1}{2}$ turns of superhelix per particle, and that the digestion pattern is consistent with the core possessing a dyad or axis of symmetry.

One further feature of the model is of interest. It has been known for some time that when closed circular DNA interacts with histones to form synthetic nucleosomes, the DNA appears to twist up to form $1\frac{1}{2}$ turns of left-handed superhelix per nucleosome (for example Germond *et al. Proc. natn. Acad. Sci. U.S.A.* 72, 1843; 1975). Since a single turn of superhelix would be too large to account for the known size of a nucleosome, various ingenious schemes have been postulated involving folds or kinks in the DNA to make it more compact. Now, however, Finch *et al.* believe that the DNA is not kinked, but that the Watson-Crick helix winds up slightly on interaction with the histones. (It is not suggested, as eager iconoclasts might conclude from the paper's abstract, that there is any change in the sense of the helix). If the histones were removed the DNA would then unwind from 10 to between 10.4 and 10.7 base pairs per turn. The fact that purified DNA fibres show exactly 10 residues per turn in the B form could arise from a distortion due to crystal packing, and Levitt (one of the paper's authors) has done energy calculations that support this view.

What next? Clearly, there is hope that better crystals will give data at higher resolution. The best crystals obtained so far have, paradoxically, come from particles in which there was considerable protein degradation. This is clearly unsatisfactory, but the authors give reasons for believing that the gross structure is similar to that of intact particles. It does not follow that intact nucleosomes do not have identical structures; little is known about why

homogeneous preparations can still refuse to crystallise, as many workers on tRNA know to their cost. In any case the present work is a promising

start, and we can look forward to following a period of intensified competition between the interested laboratories. □

Can surfaces of metals spontaneously distort?

from John Pendry

THE simplest model of atomic arrangements at clean surfaces assumes that the surface is created by removing all atoms on the vacuum side of the surface plane whilst freezing the positions of those on the crystal side. Such a model belies the complexity of forces at surfaces: structure is determined by the electronic energy levels which are known to be severely distorted by the presence of a surface, and the wonder is that so simple a model should ever work rather than that there are exceptions to it!

Deviations from the model are particularly easy to detect when they involve structure of new symmetries, that is, puckering of the surface. In that instance the diffraction pattern obtained using a beam of low energy electrons changes and acquires extra

details clearly visible on the fluorescent screen used to detect the electrons. It was in this way that two groups (Felter, Barker and Estrup at Brown University (*Phys. Rev. Lett.* **38**, 1138; 1977) Debe and King at Liverpool University, (*J. Phys. C* **10**; 1977)) working independently, discovered a structural rearrangement on the (001) surface of tungsten. A similar rearrangement on the (001) surface of molybdenum was also observed by the Brown group.

The rearrangement occurred below 300 K and the transition appeared to be perfectly reversible. In a subsequent preprint Debe and King report a determination of the space group of the new structure, and are currently analysing intensities in their diffraction pattern in order to find the detailed atomic configuration.

Although observation of a structural rearrangement is relatively simple it is no trivial matter to be sure that it was not induced by impurities. In fact hydrogen is known to induce a similar structure on a tungsten (001) surface. The present structure was probably originally observed by Yonehara and Schmidt (*Surface Sci.* **25**, 238; 1971) but the question of hydrogen contamination could not be resolved at that time. The present work establishes conclusively that the structure is a clean surface effect. The Brown group also demonstrate that it is not due to a phase transition in the bulk of the material; chromium is known to have such a transition, but molybdenum and tungsten were both shown to be free of bulk transitions by X-ray analysis which is sensitive only to bulk effects.

It has been clearly shown that the distortion is due to an intrinsic property of the clean tungsten surface but it has yet to be established which particular property is responsible.

The stable structure of a system is always the one with lowest free energy (E) so that if we plot E as a function of, say, the amplitude (d) of a periodic displacement of the surface atoms it will appear as shown in Fig. 1. Two cases are shown. In Fig. 1a the free energy has two minima which vary as functions of temperature. At the transition temperature the second minimum overtakes the first and the stable structure jumps discontinuously to one with a finite-amplitude periodic displacement of the surface atoms. For the surface to achieve the newly stable configuration many surface atoms must jump across the potential barrier between the metastable and stable structures. It is a characteristic of such transitions that they are 'sluggish' tending to show supercooling and superheating effects.

No such hysteresis was observed in this experiment and it is more likely that the transition was of the so-called 'soft mode' type shown in Fig. 1b. The restoring force for the displacement in this type of transition goes continuously to zero as the temperature is lowered to the transition point. Hence there is a precursor to the transition in the form of a vibrational mode whose frequency goes to zero, that is to say goes 'soft', as the temperature is lowered. Below the transition point the amplitude of the periodic displacement becomes finite, increasing with decreasing temperature. The period of the distortion need not be commensurate with the original lattice spacing but in the instance of the tungsten (001) sur-

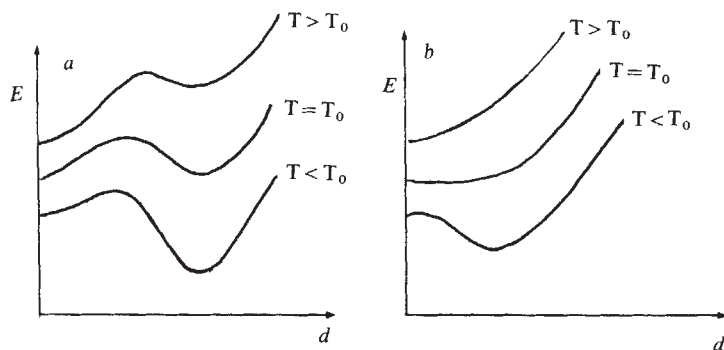


Fig. 1 Free energy plotted as a function of d the amplitude of a periodic displacement of surface atoms; a , for the case of two minima; b , for the case when a soft mode appears.

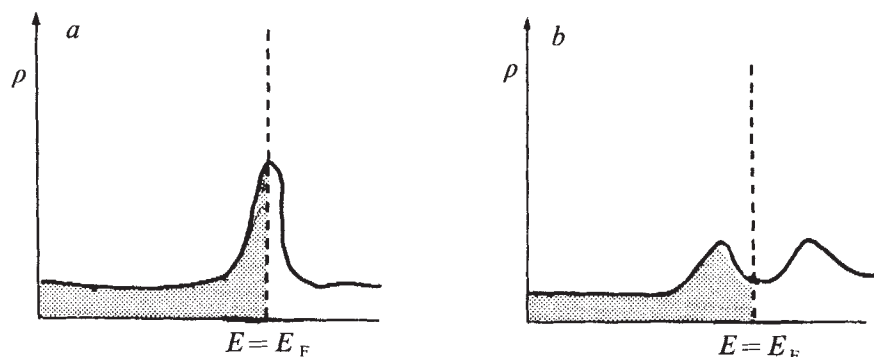


Fig. 2 a , Schematic density of electronic states with a large peak near the Fermi energy which favours a transition that splits the peak as shown in (b) , thus lowering the average energy of electrons.

John Pendry is Head of the Theoretical Physics Group at the Daresbury Laboratory.

face it appears to be so, the new periodicity being double the old one.

A common mechanism for producing soft modes is the occurrence of a high density of electronic states near the Fermi level, giving rise to a so-called Peierls instability. The argument is that distortions of lower symmetry split the electronic levels, driving some above and some below the Fermi level. Figure 2 shows the general idea. If the density of states at the Fermi level is high enough the lowering of energy in the occupied states will cancel other positive contributions and make the distortion energetically favourable.

It seems likely that a theory of the clean surface reconstruction will also shed light on chemisorption of foreign atoms. The Brown group observed that adsorption of hydrogen produces diffraction patterns that though different from the reconstructed clean surface pattern, have certain features in common with it. They speculate that hydrogen induces the surface to reconstruct even when the temperature is above the clean surface transition point. If this is correct it is to be expected that the soft mode responsible for the reconstruction will affect the energies of chemisorption.

These experiments challenge our understanding of surface electronic structure and its role in creating soft modes. Densities of states at surfaces are not easy to determine with the precision demanded here, but on the other hand as much experimental information is available about the electronic structure of tungsten as almost any other material and some interesting developments can be expected. □

Disulphide bonds and protein folding

from M. J. Geisow

THE study of the mechanisms by which proteins fold seeks to unravel the stereochemical determinants of tertiary structure, with the commendable long-term goal of predicting three-dimensional structure from amino acid sequence. Advances in the 1970s, both in formulations of concepts and in theoretical and experimental treatments of protein folding have been comparable with the rapid development of protein crystallography in the 1960s. By analogy, the next decade should see the first predicted structures, a preview of which might be the successful approximation of the pancreatic tryp-

M. J. Geisow is a member of the scientific staff at the National Institute for Medical Research, London.

Globin hnRNA = pre-mRNA

from Bob Williamson

DEFINITIVE work proving the existence of a high molecular weight precursor of mRNA has at last appeared, confirming the suspicions of a decade. Readers of these columns will know that for years the argument has proceeded between those who thought each high molecular weight RNA molecule an aggregate, and those who thought each cleavage due to inadequate purification techniques and omnipresent and omnipotent ribonucleases. Recently several groups, and in particular P. Curtis and C. Weissmann in Zurich, have shown a 15S precursor double the size of globin mRNA in induced mouse Friend erythroleukaemia cells, but this does not satisfy the true exponent of precursors. We need hnRNA molecules 10 times larger than the final product, for a given mRNA, to fit our favoured theory.

Bastos and Aviv have now provided the data (*Cell* 11, 641; 1977). They have looked at nuclear RNA from both mouse Friend cells and mouse spleen, and have found a nuclear RNA containing globin sequences at least 27S in size (approximately 5,000 nucleotides long), or seven times the mRNA length. Aviv's group identified this RNA species using a column of globin complementary DNA (cDNA) bound to cellulose. Since the cDNA was present in large excess the kinetics of hybridisation were favourable even for the small amounts of globin-specific hnRNA present.

After a 5 min pulse of labelled uridine, over two-thirds of the counts are in an RNA molecule sedimenting at 27S. It is processed with a half life of 5 min, first to an RNA of size 15S, previously shown by others, and then to the final mRNA. (There may be a small pool of mRNA in the nucleus,

which takes time to equilibrate to the cytoplasm; there is room in Aviv's data for yet another control step at this point). The scheme seems very similar for Friend erythroleukaemia cells and normal spleen, which is gratifying, since there is always residual concern when a generalisation is based on as atypical a model as a cell in continuous culture transformed by an RNA tumour virus and induced into erythropoiesis by an organic chemical acting on membranes.

The other interesting result presented is that the large 27S precursor has no poly(A) tail; this only appears after cleavage to the 15S molecule. Therefore poly(A) addition is not only a post-transcriptional event; it also occurs as part of intermediate processing. It should be possible shortly to determine whether the poly(A) is added internally and what is the position of the mRNA sequence in the 27S precursor.

Another group has also studied mouse spleen cells in culture using DNA bound to cellulose, and using very rigorous denaturation conditions with treatment by formaldehyde, which breaks secondary structure irreversibly (Kwan, Wood & Lingrel *Proc. natn. Acad. Sci. U.S.A.* 74, 178; 1977). In this case the RNA, pulse labelled for 10 min and prepared by several different techniques, was isolated and run on gels, and showed the 15S precursor peak but no 27S RNA species. The reason why some groups fail to find this larger species while others do is unclear, but may be related to the very rapid processing of nuclear RNA, even while it is synthesised, in some culture conditions.

Bob Williamson is Professor in the Department of Biochemistry at St Mary's Hospital Medical School, London.

sin inhibitor structure by Levitt and Warshel (*Nature* 253, 594; 1975).

Many reports of the change of protein structure consequent upon denaturation and refolding have been based on kinetic measurements using optical, chiroptical or magnetic resonance properties of the amino acids to monitor the microenvironment. Typically, these experiments indicate biphasic or multiphasic pathways, which point to the existence of significant intermediate states. Thermodynamic observations are often more simply explained by an equilibrium of native and unfolded forms, suggesting

cooperativity in protein folding. Such a hypothesis is almost certainly an oversimplification for whole proteins, but might well describe the formation of component elements of secondary structure such as β -pleated sheet. The pattern of formation of hydrogen bonds in this structure would seem to provide an excellent physical model for a highly cooperative folding process.

The classical method of studying mechanism in chemistry is to trap intermediate states and infer the pathway from the structures of these intermediates. In disulphide proteins nature has provided just such potential inter-

mediates, since the order of formation of disulphide bridges must reflect the folding pathway. 'Freezing' the reaction by acid or alkylating reagents then provides covalent intermediates for study at leisure.

A prerequisite for the extrapolation of results from the particular disulphide proteins studied to general notions of folding, must be the demonstration that disulphide formation does not provide of itself the dominant driving force for protein folding. The consensus is that the peptide chain promotes rapid and specific disulphide formation and not *vice versa*. For example, in denaturants such as guanidinium chloride, correct disulphide bridging does not occur, even in the presence of thiols to allow disulphide exchange to correct 'wrong' bridges.

Having said this, it is also certain that in the absence of denaturants, the initial disulphide bridges formed are not necessarily native. Workers usually refold proteins in the presence of redox systems such as dissolved O_2 , oxidised and reduced glutathione or even disulphide exchange proteins (reshuffleases) to prevent the accumulation of non-native forms. In all of these oxidation methods it is clear that thiol-disulphide exchange is the principal process which leads ultimately to the native forms. Since a third factor (an oxidised thiol) must participate in the formation of each disulphide bond a question of accessibility arises, particularly for disulphide bridges which are deeply buried in the final structure. The degree to which the oxidative mechanisms influence the folding pathways is not known, but many workers have nevertheless found it relevant to ask what light disulphide bridges can shed upon the folding process.

The questions then are: which are the initial disulphides, what regions of tertiary structure do they connect and are there early native disulphides or do they consist of predominantly non-native forms? The principal answers to these questions have been sought in studies of ribonuclease A (RNase A), bovine pancreatic trypsin inhibitor (BPTI) and lysozyme. These are arguably an unrepresentative group because they are all very small and stable proteins, but they are sufficiently well characterised for precise study.

Two general mechanisms have emerged. The first is a random search mechanism in which a rapid phase occurs where there is formation of many randomly-paired disulphides and mixed disulphides, followed by a reshuffle to the native form. This folding pattern was deduced for RNase A (Hantgan *et al.* *Biochemistry* **13**, 3421; 1974).

The second is a limited search mech-

anism where there is rapid formation of a few specific disulphides, followed by slower reoxidation and exchange of the remainder to produce the native structure. Anderson and Wetlaufer (*J. biol. Chem.* **251**, 3147; 1976) found that the first two native disulphides of reduced lysozyme formed eight times more rapidly than the second two, implying an ordered progression to the final structure. Creighton (*J. molec. Biol.* **95**, 167; 1975) observed that the refolding of BPTI could only take place through the formation of two of the four major disulphide intermediates present initially, suggesting an obligatory folding pathway.

The latter mechanism is certainly more consistent with the idea of nucleation regions which, formed early during folding, predispose to particular disulphide bonds. Creighton notes that the predominant cysteine residues involved in early disulphides in BPTI are at the centre of an α -helix and an antiparallel β -pleated sheet respectively; secondary structure is the favoured site of nucleation in folding proteins. Even if each of these mechanisms is correct for the respective protein, the experiments rule out the 'all or none' or co-operative picture of protein folding, since disulphide formation is far more rapid than the return of biological activity.

More recently an ingenious thermodynamic approach has overcome the technical problems of 'trapping' the rapidly reoxidising disulphide bridges. Acharya and Taniuchi (*Proc. natn. Acad. Sci. U.S.A.* **74**, 2362; 1977) have allowed randomly alkylated lysozyme containing one blocked cysteine residue per mol of protein to refold over an extended period. Enzymically active material isolated after refolding contained eight isomers representing each of the four possible ways of forming three native and one open disulphide bridges. There were similar amounts of these four forms. Such an observation clearly rules out an obligatory pathway for the formation of correct disulphide bonds, since the folding routes must have been independent of each disulphide in turn, although ultimately producing near-native molecules. Acharya's and Taniuchi's result does not fix the sequence of events leading to the correct structure and therefore cannot rigorously distinguish between a random or nucleation folding mechanism. However, the inability of a particular disulphide to form did not appear to influence the final fold. This lends some support to the notion that the early folding events reflect what the peptide chain is trying to do, rather than simply the need of the cysteines for oxidation. The truth, of course, is probably a bit of both.

The recent topological analysis of protein structure by Levitt and Chothia (*Nature* **261**, 552; 1976) points to the presence of a limited set of folding units in globular proteins, composed of adjacent elements of α -helix and β -structure. The elements of secondary structure concerned are also close in linear sequence, suggesting that they could quickly form appropriate interactions to produce units of tertiary structure. Such folding units apparently occur with a statistically very significant frequency in the 31 proteins examined. The authors argue that the folding of proteins is kinetically determined by the high probability of formation of these folding units, which subsequently interact with each other to produce the native structures. This attractive hypothesis might be testable in disulphide proteins. The identification of cysteine residues which would be brought into proximity within a Levitt-Chothia folding unit should coincide with the experimentally observed early disulphides, even though these might not be the final native ones. □

Weather on Neptune

from Garry E. Hunt

STUDIES of the weather systems of planetary atmospheres provide a greater insight into the meteorological processes involved in a wider range of conditions than can be achieved through investigating the Earth alone. In addition to the direct spacecraft observations, Earth-based measurements of the changing cloud patterns of Venus, Mars, Jupiter and Saturn have provided a wealth of data on their atmospheric phenomena. The same approach cannot be applied to atmospheric studies of Uranus and Neptune since these planets are too distant for direct telescope observations of any cloud systems to be possible. In these conditions it is necessary to adopt a subjective approach such as monitoring the planetary brightness spectrum or changes in the absorption spectra formed in the cloudy planetary atmosphere. Certainly patrols of the hydrogen features in the Jovian atmosphere have been shown by Hunt and Bergstrahl (*Icarus* **30**, 511; 1977) to be good identifiers of planetary motions and variabilities in the cloud layers. A patrol of the Neptune reflectance by Joyce *et al.* (*Astrophys. J.* **214**, 657; 1977) indicates a substantial but unexpected increase in the planet's reflectance between 1 and 4 μ m, between April 1975 and March

Garry Hunt is in the Department of Physics at University College, London.

1976, with an increase of a factor of 4 at $3.5\ \mu\text{m}$ (L band). Pilcher (*Astrophys. J.* **214**, 663; 1977) suggests that these measurements are the first direct evidence of weather on Neptune caused by rapid variations in the cloud structure. Paradoxically, the simultaneous observations of the nearer planet Uranus show no variation during this observational period. Could Neptune have weather and Uranus not?

The radiative time constant is the time taken for a mass of air to warm up or cool by radiating in the infrared portion of the spectrum. Stone (*Space Sci. Rev.* **14**, 444; 1973) suggests that for Uranus this parameter is about 600 Earth years which is long compared with both its orbital and rotational periods of 84 yr and 22 ± 2 h. Similarly for Neptune the relaxation time is about 2,200 yr which is again enormously long compared with the orbital periods of 164 yr and 21 ± 2 h respectively. Consequently, this would suggest minimal diurnal and seasonal changes in the atmospheric structure of the visible cloud layers. Furthermore, we would expect the planets to behave in a similar manner, which, at first sight, is not borne out by the measurements of Joyce *et al.* (*op. cit.*). Is it possible for Neptune to possess a more active weather system or more variable cloud layers?

The increase in the Neptune reflectance observed by Joyce *et al.* must be due to a corresponding increase in the planetary albedo. Correspondingly, it would also require a reduction in the atmospheric absorption, which is substantiated by the smaller amounts of methane and hydrogen they derive, compared with the previous studies by Owen (*Icarus* **6**, 108; 1967) and Encrenaz *et al.* (in *Exploration of the Planetary System* ed. Woszczyk, A. & Iwaniszewska, C., Reidel, 1974) respectively. Does this imply a variable cloud structure affecting the level where the absorption lines are formed?

There seems little doubt that the Neptune atmosphere (and Uranus too) will possess layers of cloud. Prinn and Lewis (*Astrophys. J.* **179**, 333; 1973) predict a layer of methane haze in the upper troposphere of both these planetary atmospheres, which may be more substantial in thickness in the colder Neptune environment. At the lower levels, layers of NH_3 , NH_4SH and aqueous NH_3 could possibly form but they are unlikely to affect the spectroscopic or photometric observations. What could cause the methane cloud opacity to vary, since this is the most likely candidate to explain the paradoxical observations of Joyce *et al.*?

Morrison and Cruikshank (*Astrophys. J.* **179**, 329; 1973) found that at $20\ \mu\text{m}$ Neptune has a higher brightness

temperature ($\sim 58\ \text{K}$) than Uranus ($\sim 53\ \text{K}$). Does this mean that Neptune (and not Uranus) may have an internal heat source? It would then be possible for this energy source to produce convective activity in the Neptune atmosphere which would be important in the planet's meteorology. The solar energy at the distance of Neptune is extremely weak, and would therefore have a limited effect in suppressing the atmospheric convection, as is apparently achieved in the region of Jupiter bounded by $\pm 45^\circ$ latitude (Ingersoll *Space Sci. Rev.* **18**, 603; 1976; Hunt *Adv. Physics* **25**, 455; 1976). It would then be possible for this redistribution of atmospheric heating to modify the upper troposphere thereby affecting the condensation processes. The resulting variability in the cloud structure would then create the changes in reflection and absorption properties observed. However, these measurements are for the whole planetary disk, so that it is possible that global scale features are being observed. Perhaps this then suggests that Neptune may not possess belts and zones which are so familiar on Jupiter and Saturn.

But why should Uranus not show similar variations during this time period? The $20\ \mu\text{m}$ observations of Morrison and Cruikshank (*op. cit.*) could be interpreted as suggesting that Uranus may not have an internal heat source. This would then imply less convective activity in the Uranus atmosphere, so that perhaps one would not expect to observe variability in the cloud layers. But perhaps Uranus does have an internal heat source. Then it is quite likely for the atmosphere to exhibit some variability, but the time scale may be affected by peculiar inclination of the rotational axis. The level of activity may depend upon the portion of the disk that is observed, so that it is essential for a patrol of the Uranus atmospheric features to be carried out during the next few years.

The brightness of the outer planets, Uranus and Neptune have been patrolled by Lockwood (*Science* **190**, 560; 1975) to study possible variations in the solar constant, which would obviously affect every planetary atmosphere. If these planets, Uranus and Neptune, possess variable cloud layers, which could modify the planetary brightness, then it may be necessary to reconsider this type of study. However, there is little doubt that future studies of the meteorology of Neptune provide a further environment to study atmospheric processes very different from those of the Earth's atmosphere, so that there is a great deal to be done with our telescopes and computers before the planet is visited by Voyager in 1989. □

Structural gene for myosin

from Carol K. Klukas

IN a recent issue of the *Journal of Molecular Biology* (**114**, 133; 1977) A. R. MacLeod, R. H. Waterston, R. M. Fishpool and S. Brenner report their identification of a structural gene for a myosin heavy chain in *Caenorhabditis elegans*, a small nematode worm. Their report represents another step in the long term project being undertaken in Sydney Brenner's laboratory to dissect the genetic specification of this creature, a representative and yet manageable eukaryotic organism.

The basic approach is that used to dissect the much simpler genetics of bacteria. In bacteria genetic analysis of mutants provides an outline of the genetic organisation: how many genes are involved in any particular process and how they are arranged on the chromosome for example. Biochemical measurements (such as assays for activity of specific enzymes in various mutants) then can provide information on precisely how the genes work together to specify a particular biosynthetic process within the bacterial cell.

But the problem of deciphering and dissecting the genetic system which dictates the complexities of a multicellular organism, in which different cells each with their particular biochemical processes, must be positioned in a correct spatial relationship and must function in a correct relationship temporally, seem daunting. However, the 1-mm long *C. elegans*, which consists of a total of only some 600 cells and has a life cycle of only $3\frac{1}{2}$ days is an organism uniquely suited to such an attempt.

The DNA content of this worm's genome is only 20 times that of *Escherichia coli*, and of this DNA only 6.7×10^7 base pairs code for unique sequences, enough to code for about 6.7×10^4 average-sized genes. This number is large enough, but is considerably more manageable than that of most other eukaryotes. Furthermore, because *C. elegans* is hermaphrodite, recessive mutants are easy to isolate and since the defect is reliably transmitted to the offspring a ready supply of recessive mutants for study is assured. Although *C. elegans* does not have many external features in which abnormalities can be detected, useful behavioural mutants can be quite easily detected visually whose

Carol Klukas is in the Department of Biophysics at King's College, London.

abnormalities often directly indicate mutations in the nervous system or musculature.

With these advantages in mind, Brenner's group has set about the slow task of dissecting the genetic specification of *C. elegans*, especially that of the nervous system. The work involves the characterisation of mutants such as E675 which maps in the *unc-54* gene and was used in the recent *J. molec. Biol.* paper to identify the *unc-54* gene as the structural gene for a myosin heavy chain present in a major fraction of the total nematode myosin. All worms with mutations in the *unc-54* gene, including deletion mutants, have disorganised muscles due to a drastic decrease in the number of thick filaments in the body wall musculature, but these mutants survive because the pharyngeal muscle continues to function normally. In E675 the myosin has a molecular weight of 2×10^5 rather than the normal 2.1×10^5 component. By means of cyanylation and cyanogen bromide cleavage the authors show that the mutant heavy chain is an altered form of the wild-type myosin

and thus conclude that *unc-54* is the structural gene for this heavy chain.

There are always some thick filaments visible in the body wall cells of *unc-54* mutants, even in the deletion mutants. This indicates the presence of multiple myosins in *C. elegans* as has been noted in higher organisms. In normal worms do other myosins associate with *unc-54* heavy chain to form hybrid myosin molecules or do thick filaments containing mixed myosin populations occur? The suggestion from observation of mutants is that mutant heavy chains can interfere with the production of normal myosin in some way and it will be part of the *C. elegans* puzzle to work out this interplay between myosins and the genes which direct their synthesis.

The whole puzzle is very complex indeed, but each piece which is fitted in by Sydney Brenner's group brings closer a complete picture of just how the information specifying a higher organism is stored in its genes and unravelled in the correct sequence to dictate the development of an organism such as *C. elegans*. □

plate carrying the South Asian sub-continent moved northward and collided with the front edge of the Eurasian plate. It obliquely compressed and in stages ploughed into the ancient Asiatic continent, and in fact is still moving at a velocity of $\sim 0.5 \text{ cm yr}^{-1}$. Palaeomagnetic measurements suggest that the suture of the two plates is located roughly along the Yalu-Tsauo valley.

Gravity and earthquake data suggest that the thickness of the Earth's crust at Mt Jolmolumma (Mt Everest) is $\sim 48 \text{ km}$, much less than that in the vicinity of Lhasa which is $\sim 70 \text{ km}$. Geophysicists found, on the other hand, intense hydrothermal activity along a belt south of the Gangdise Range and north of the Himalayas. The initial interpretation is that this widespread geothermicity is the result of lava activities.

Data were collected along built and planned highway routes, on the occurrence and development of mud-and-stone flows. Glaciation was studied over large areas. Chinese glaciologists are now working out laws of motion pertaining to a rare oceanic (warm) type of glacier, the distinguishing features between this type and the continental (cold) type, and the changing distributions of both on the Plateau. Wave-like glacial movements were also observed for the first time. After long years of slow movement, some glaciers show a sudden burst of speed in their advance. The momentum change is frequently so large that the middle moraines on the surface of the ice are twisted and the ice surface shows cracks like a spider's web.

It is reported that the large quantity of good data are subjecting to severe tests many views currently adopted in the West. For instance, one hypothesis maintains that the Plateau was totally glaciated during the Quaternary Period and that few plants and animals escaped extinction to survive today. The survey, however, indicates that the Tibetan flora and fauna consists of many species, some of which are left over from the Tertiary Period. Tibet has more than 4,000 species of higher plant. Geological data show that during the glacial period in the Quaternary, the glaciers were indeed much larger than at present. At that time the Himalayas could not yet completely block winds coming from the Indian Ocean, and precipitation in most parts of Tibet was greater. However, data indicate that the Tibet Plateau has never been completely covered by glaciers. Another common belief in the West is that northern Tibet is in a drought belt. The survey establishes that the vegetative cover there is basically the grassland type

Surveying the roof of the world

from T. B. Tang

MANY large-scale geographical and scientific surveys have been conducted in China since 1949, particularly in the past five years. In 1973-75, for example, the State Department of Agriculture and Forestry undertook a land resources survey of the Heilungkiang (Amur) region, in which scientists co-operated with the ordinary citizen. The Department of Geology carried out in 1974-77 an airborne geophysical survey of the southern Yellow Sea, East China Sea, and South China Sea. The largest and most comprehensive survey to date must be, however, the four-year expedition to the Chinghai-Tibet Plateau, organised by the Academia Sinica and started in 1973. The Plateau, sometimes referred to as the 'third Polar Region' or the 'roof of the world', is of immense scientific and other interest. In 1951 and 1960, local and specialised surveys were carried out in that vast area, and their results were used in the planning of the present expedition. More than 400 scientists, from 45 institutes and specialising in some 50 disciplines, took part at one time or another. The field work now completed, the programme is entering the stage of laboratory analysis, data processing, synthesis of

results, and theoretical interpretation. In June the Academia Sinica arranged a conference at which selected fossils, photographs and films were shown, and papers describing preliminary results were discussed (*Hsinhua News Agency* and *Ta-kung-pao*).

It has long been known that the Plateau used to be under sea-level, but little was ascertained as to exactly when and how the uplift occurred. That during the early and middle Jurassic period Tibet was a shallow sea or depression was confirmed by the present survey from palaeontological evidence. More than 6,000 fossils have been collected, dating from the Proterozoic and Cambrian periods onwards, and including those of a Hipparion (exhibited at the conference) and a sauropod dinosaur. It is the first time that dinosaur fossils have been found at places now at such a high altitude. (A preliminary regional stratigraphical map has been published.)

In the Pliocene Tibet became low-lying dry land with subtropical conditions. Then, in the ensuing period of several million years, a tremendous uplift took place. The geophysical model most discussed at the conference proposes that the upheaval was generated mainly by the spreading of the Indian Ocean floor, so that the

which is, or can be made to be, good grazing.

Water resources have also been investigated. The survey determines that Tibet has 1,000 lakes, about half of the total in China. Most are tectonic lakes, the rest mainly formed by glaciation. They contain abundant deposits of salt, soda, sodium sulphate, and borax. The lakes have significant effects on the local climates and the areas around them possess great potential for agriculture and animal husbandry. The fish in them are chiefly Schizothoracinae of the Cyprinoid family and barry loaches of the Cobitidae. They are different from those on the plains, preserving many of their primitive features. It is thought that they have evolved from those existing in the late Tertiary Period, and have undergone some adaptive changes during the uplift of the Plateau.

The Chinese collected over 50,000 specimens of higher plants, and over 70,000 of insects, birds, animals, fish and other aquatic life. Preliminary identification work has revealed more than 100 new species of plants, some presumably having developed from known species (during the upheaval)

and some being entirely unique to the Plateau. Identification of the detailed natural-geographic zones is now at an advanced stage. Incidentally, as a result partly of the survey but mainly of research started in 1971 by the Chinghai Institute of Biology, a Tibetan medical pharmacopeia was published in July. It is in three volumes, the first two dealing with herbal drugs and the third with animal products having therapeutic applications. 455 drugs are listed, an outstanding example among which is saussura, a herb with a white pappus, of great medicinal value, but scarce in other parts of China.

Besides providing a wealth of unique scientific data, the survey has located many important mineral, water, geothermal energy, and other resources, and showed that the Plateau possesses agricultural and cattle-raising potential. No doubt the development of Tibet will continue at an ever increasing pace. An oil pipeline is already in existence from Golmo in Chinghai to Lhasa, and work has started on a 2,100-km railway line to link up with the national network.

Stress physiology of crop plants

from Hamlyn G. Jones

An international conference on Stress Physiology of Crop Plants sponsored by the Boyce Thompson Institute for Plant Research and the Rockefeller Foundation was held at the Boyce Thompson Institute, Yonkers, New York, on 28–30 June, 1977. The conference was organised by H. Mussell and R. C. Staples.

It is the tropical countries which have the greatest potential for providing the extra food which will be necessary to feed the growing world population, since they have extensive areas of potentially cultivable land where crops either are not grown at all or only yield poorly because of stresses such as drought, non-ideal temperatures or adverse soil conditions. This was the message in the first paper of the conference, given by M. N. Christiansen (USDA, Beltsville).

In the past, much of the effort towards improving crop yields in these situations has involved the breeding of high yielding varieties suitable only for near optimal conditions, with the consequent need to ameliorate the stresses by installation of expensive irrigation systems or by using high inputs of fertiliser or pesticide. As was

pointed out by R. C. Staples (Boyce Thompson Institute) in his introductory talk, this approach has led to the poorest farmers often benefiting least from the 'Green Revolution'. Following up this point, F. N. Ponnamperna (IRRI, Philippines) emphasised the tremendous potential for increasing food production without requiring large increases in inputs which is offered by the breeding of new varieties tolerant of stress. He gave examples of the recent advances achieved by the International Rice Research Institute in breeding lines tolerant of soil conditions such as salinity, alkalinity and zinc deficiency.

The conference was significant in that it was probably the first major attempt to review progress in the application of fundamental plant physiological research to the breeding of crop varieties adapted to a range of stressful environments. Although a wide range of stresses including disease, aluminium toxicity and air pollution was covered in the meeting, the majority of papers were concerned with temperature or drought.

Among several interesting contributions was one by M. J. Jaffe (Ohio University, Athens, Ohio) on a commonly neglected stress. He reported a series of studies on the effect of mechanical stimulation, such as might be experienced by plants brushing against one another in the wind, on plant growth and development. Even slight rubbing of expanding internodes was shown to reduce their extension dramatically. G. F. Somers (University of Delaware, Newark) described some speculative work where irrigation with sea water is being used in a programme designed to select new salt-tolerant crops from among wild species native to salt marshes. If successful, this approach alone could greatly increase the world's arable area, since there are large areas with saline soils or where saline irrigation water only is available.

The general problems of improving crop tolerance of stress were illustrated by the contributions in the field of drought resistance. These ranged from reports of fundamental studies at the molecular level to reports by plant breeders actually concerned with breeding new varieties. The present state of the art in breeding for drought resistance in wheat was outlined by F. T. Townley-Smith and E. A. Hurd (Agriculture Canada, Saskatchewan), who described the techniques adopted in their own programme. Although they make some selections on the basis of 'physiological characters' such as the rate of wilting of cut leaves or the length of roots in seedlings, their programme relies heavily on yield measurement as the main selection criterion. Physiologically based screen-



A hundred years ago

With reference to Galileo's claim to be the inventor of the telescope, M. Wolf quotes (*Annalen der Physik und Chemie*) from a manuscript of Scheiner (1616) in a library in Zurich, a curious passage, of which the following is part: "It must be allowed first, considering what the telescope does, that Baptista Porta has better right to be thought the inventor, because he describes, after his own way, in obscure words and puzzling expressions, an instrument like the telescope. But secondly, if we speak of the telescope, as it is now used after general perfection, we must say that neither Porta nor Galileo is the first discoverer of it, but the telescope in this sense was discovered in Germany, among the Belgians, and that accidentally by one Krämer, who sold spectacles, and either for amusement, or experimentation, combined concave and convex glasses, so that with both glasses he could see a quite small and distant object large and near; at which success being rejoiced, he united several similar pairs of glasses in a tube, and offered the combination at a high price to wealthy people. Thereafter they (the telescopes) became gradually more common among the people, and spread to other countries. In this way two of them were brought for the first time by a Belgian merchant to Italy.

from *Nature* 16, 30 August, 390; 1877.

Hamlyn G. Jones is a Lecturer in Botany at the University of Glasgow.

ing is not emphasised in their programme, partly because the tests tend to be too complex to use on large numbers of lines and partly because most such tests tend to be poorly related to field performance. This paper highlighted the somewhat depressing fact that, to date, the physiological approach has not made much contribution to the development of new stress-tolerant varieties. In spite of this there was general agreement that really large and rapid improvements in stress tolerance will only come about by cooperation between breeders and physiologists.

Part of the difficulty is that in the past many laboratory physiologists have been reluctant to face the complexities of the field situation, which is, after all, where their ideas must ultimately be tested. An encouraging feature of the conference was the surprisingly large number of contributions from workers who have been facing this challenge and have begun to study stress physiology in the field and to apply this work to plant breeding. N. C. Turner (CSIRO, Canberra), for instance, reviewed recent work on the mechanisms whereby plants adapt to drought stress in the field. The important message was that the responses observed in the field were frequently quite different from those found in controlled environments so that much laboratory work may be misleading.

It was apparent from the various papers and discussions during the meeting that we are further advanced in our understanding of and ability to breed for tolerance of some stresses such as cold, specific ion toxicities or more particularly disease, than we are for drought. This may be largely a function of the complexity and variability of droughts and the range of possible tolerance mechanisms. Surprisingly, perhaps, those such as A. Blum (Volcani Institute, Bet Dagan, Israel) and C. Y. Sullivan (USDA, Lincoln, Nebraska) who work in the most arid regions, tended to be the most optimistic and appeared to have made most progress in developing physiologically-based screening tests for drought resistance. Sullivan, for instance, reported good correlations between field performance of sorghum and several physiological tests including the ability of disks of leaf tissue to withstand osmotic stress or short periods at high temperatures.

The basic optimism and philosophy both of the conference organisers and of the vast majority of participants is probably best summed up by this extract from the 1976 annual report of the Boyce Thompson Institute: 'We believe that there is no more important task for research to undertake than

providing the technology to feed (and clothe) the continually expanding world population, . . . and that understanding plants and pests at the basic physiological and biochemical level is essential for the future breakthroughs necessary. . . .'

Earthquake prediction

from R. P. Adams

Discussions on earthquake prediction remain one of the drawcards at seismological meetings. It was appropriate that on 16 August a full-day symposium was devoted to this topic at the General Assembly of the International Association of Seismology and Physics of the Earth's Interior, being held at Durham.

INTERNATIONAL interest was evidenced by the fact that authors from seven different countries contributed the fifteen formal papers. It was fitting that three papers were given by scientists from the People's Republic of China, as this was their first chance to appear at meetings sponsored by the International Union of Geodesy and Geophysics since China's admission to that body 10 days earlier.

In coming from the only country yet to have successfully predicted major earthquakes, the Chinese have a clear lead in this field. Their first paper, presented by Chang Yu-ming on behalf of a group of 10 authors, discussed in detail the general seismotectonics of China, while two later papers dealt with detailed descriptions of precursory phenomena associated with known earthquakes. Tung Sung-sheng spoke of variations in seismic velocity in Yunnan Province that enabled a general warning to be issued a year before two earthquakes of magnitude 6.7 and 6.4. Chen Yun-tai reported observed changes in gravity measurements of up to 350 μ gal before and after the successfully predicted Haicheng earthquake of 1975, and smaller gravimetric changes associated with the catastrophic Tangshan earthquake of 1976, that was not predicted. It was suggested that these surprisingly large differences could have arisen from the movement of ground water. In the general discussion it emerged that although the immediate warnings before earthquakes were usually dependent on the occurrence of foreshocks, there has been at least one

case in Yunnan Province where this was given by other methods, such as variations in measurements of radon concentration in ground water, and resistivity.

Little was said about short-term precursors, but a new concept appears to be emerging for identifying 'precursory swarms' as a long-term indicator. This idea was most fully developed by F. F. Evison (Wellington) who elaborated an idea first published in *Nature* in April 1977. Evison's hypothesis is that a swarm of activity near the area of a forthcoming earthquake is followed by a 'precursory gap' which lasts until the occurrence of the 'major event'. As well as the position of the impending earthquake, its time of occurrence and magnitude are also indicated by the pattern of seismicity. It is still difficult to identify and interpret such swarms, and different criteria may be needed in different areas.

V. I. Keilis-Borok (Moscow) also spoke about the recognition of seismicity patterns before impending earthquakes. He postulated criteria for determining periods of 'activation' and 'quietness', rather analogous to Evison's concept, and also referred to precursory earthquake swarms. In reporting a detailed study of seismicity in the Aleutian Arc, Engdall and Kisslinger (Boulder, Colorado) also commented that they had observed similar patterns, and precursory variations in seismicity were observed by Talwani (South Carolina) in small earthquakes associated with a reservoir.

Other papers covered as diverse topics as the tilting of the Hawaiian Islands in relation to large circum-Pacific earthquakes, (Berg and Sutton, Honolulu), the prediction of intermediate-depth Romanian earthquakes by assigning time-series to historical events (Purcaru, Frankfurt), and the theory of deciding what constitutes an 'anomalous' reading (Jackson and Ergas, Los Angeles). It was also pleasing to note speakers presenting what seem to be negative results. Susan Raikes (Pasadena) reported a very thorough study of P-wave residuals in Southern California, revealing no anomalies. Dolbilkina and others reported that Soviet workers also often failed to detect precursory velocity anomalies, but a small change was found by a refraction survey near a large earthquake in Kamchatka.

Earthquake prediction still awaits a major breakthrough. It now seems however, that the best prospects remain the study of seismicity patterns, with the new concept of precursory swarms for the establishing of long-term predictions, and a fuller understanding of methods used by the Chinese for the issuing of final and short-term predictions.

Robin Adams is Superintendent of the New Zealand Seismological Observatory.

review article

Decision making in animals

D. J. McFarland*

Animals must make decisions about when to feed, when to court, when to sleep, and so on, in such a way as to maximise as far as possible their chances of survival and reproductive success. It is possible to formulate in mathematical terms the optimal strategy for an animal to pursue. The theoretical optimum behaviour can be compared with the actual behaviour of the animal, and perhaps shed some light on the evolution of behaviour.

At any particular time there are many activities in which an animal could engage: feeding, courtship, sleep, and so on. It is usually impossible for an animal to carry out such different activities simultaneously, because the required movements are mutually incompatible. There must be some process or mechanism that determines which activity is to have priority at any particular time, and such a process is conveniently termed a decision process.

The decisions made by animals may be decisions on whether to continue with current activity, or to change to some other form of behaviour. They may also be decisions between alternatives that present themselves anew. In each case, the animal has to assess a wide variety of factors. Consider, for example, the situation of a herring gull (*Larus argentatus*) incubating its eggs. Normally it will sit tight until relieved by its mate. Both parents incubate the eggs, and a nest left unattended is soon subject to predation by neighbours. It is important, therefore, that the sitting bird should not leave the nest before being relieved by its partner. This rarely happens under normal circumstances, unless the bird is flushed from the nest by a predator, such as a fox or a human. Studies of herring gulls, at the South Walney Nature Reserve in Cumbria, have shown that the non-sitting member of a pair is often foraging for food, or resting on the beach. It normally returns to the territory within a few hours of leaving. Sometimes, however, the return may be delayed as a result of some mishap, injury, or capture by a scientist. The sitting bird, with increasing hunger, should eventually decide to desert the nest, because herring gulls breed in many successive seasons and it may not be in the genetic interests of the individual to endanger its life for a single clutch of eggs. The timing of such decisions is a question that has interested students of animal behaviour in recent years.

The basic dilemma for the incubating gulls lies between the possibility that its partner may return at any time, and the increasing necessity to obtain food. The situation is complicated by the fact that the gull must assess the returns on time invested in foraging, which vary considerably with the weather, the state of the tide, and the time of day. Moreover, individual gulls tend to specialise in particular types of foraging, so that the sitting gull should take account of its own foraging skills in assessing the likely returns from time spent foraging in different places.

Two questions posed by this example are of particular interest to ethologists: (1) how can we account for the decision processes involved? (2) how good are animals at making decisions of this complexity? The basic approaches to these questions stem from

decision theory, on the one hand, and the theory of natural selection, on the other. In recent years there has been some convergence between these two lines of thinking.

Decision theory

The study of decision making provides a meeting point for many disciplines: statistics, economics, philosophy, psychology and control engineering. In classical decision theory there are two important classes of variable. One, the subject's evaluation of the relative attractiveness of alternative choices, is called the utility. The other, called the (subjective) probability, concerns the subject's evaluation of the consequences of making each choice. Thus a man may wish to make a decision between going to a restaurant or to a cinema, in terms of the utility of each. He may also take account of the probability of the restaurant being expensive, or the film being poor.

Reports of attempts to investigate these elements of decision making make up a voluminous literature, but only recently has this work become directly relevant to animal behaviour.

The empirical approach to the relationship between decision theory and animal behaviour was initiated by Logan¹, who studied rats living in a box in which they had to work for any water they received by pressing a bar. The animals had to work on some specifiable terms for the water rewards. The terms were manipulated by varying the force required to depress the bar, the number of times the bar had to be pressed to obtain a reward, and the size of the reward. Logan found that the rats were sensitive to the details of the terms, in that daily water intake was reduced when any of the three aspects of the terms was increased. In later experiments, Logan² gave rats a choice between a large food reward following a small delay, and a small reward following a large delay. He was able to establish equivalent combinations that were described by 'indifference' functions, and by equations describing the relative incentive value of different amounts and delays of reward. He also gave rats a choice between constant food reward and a varied amount, or varied delay, of reward; thus introducing the concept of 'subjective probability' for the rat. In general, Logan's results were similar to those that would be expected by classical decision theory. The evidence subsequently obtained by psychologists^{3,4} and ethologists⁵, although often confounded by measurement problems⁶, tend to support the view that animals are capable of consistently evaluating alternatives and of maximising their combined value. Consideration from first principles⁷ gives rise to similar conclusions, but draws attention to some formidable problems. A simple everyday example may serve to illustrate these.

There is widespread agreement that an understanding of decision making can be gained from (1) the formulation of a

*Department of Zoology, University of Oxford, South Parks Road, Oxford, UK.

maximising principle, (2) the recognition that there will inevitably be some trade-off among various aspects of the problem, which will necessitate (3) the formulation of an optimality criterion. As an example, let us consider a university committee set up to review applications for a lectureship. Their maximising principle is to choose the best candidate, but the requirements of the post are such that there will inevitably be a trade-off between the teaching experience T and the research ability R of any given applicant. Suppose that the committee arrives at an agreed short list of two applicants A and B, and that they are able to score these for teaching and research, so that for applicant A, $T = 9$ and $R = 1$; while for applicant B, $T = 2$ and $R = 7$. Any assessment of an applicant's overall strength of candidature must rest on the optimality criterion used to evaluate teaching experience in relation to research ability. For example, if the criterion implies that strength of candidature $C = T + R$, then applicant A would score $C = 9 + 1 = 10$, while for applicant B, $C = 2 + 7 = 9$. On the other hand, if we let $C = T \times R$, then A scores $C = 9 \times 1 = 9$, while B scores $C = 2 \times 7 = 14$. Clearly, by altering the optimality criterion, without changing the scores, it is possible to come to a different conclusion as to which is the best applicant.

We now have to ask what factors influence the optimality criteria. In the university context, factors such as government attitude, financial pressures, and so forth, are likely to determine policy concerning the balance between teaching and research. As far as the selection committee is concerned these are ecological factors. An overriding influence will be the

climate of ideas concerning the maximising principle. While in some universities the best candidate is seen in academic terms, in others it may seem to appoint the youngest candidate, the most politically conformist, and so on.

Translation of this example into terminology relevant to animal behaviour is not difficult at a superficial level. For example, let us suppose that there are two main factors contributing to feeding tendency: degree of hunger, in terms of physiological requirement for food, and strength of food cues, in terms of the animal's estimate of the availability of food. There will be an optimality criterion combining these into an overall feeding tendency, similar to that combining teaching and research abilities into an overall strength of candidature. Any tendency to feed has to be weighed against tendencies for other types of behaviour, because it is important that the criteria determining the feeding tendency be related to the animal's needs as a whole. We should therefore expect the decision criteria for feeding to be shaped by natural selection in accordance with the animal's ecological circumstances. For example, where food availability is erratic, more weight should be given to cue strength, while the weight attached to hunger should be related to the animal's physiological tolerance. We now come up against a problem that has dogged animal psychology for many years. While there has been a great deal of research on specific 'drives', such as hunger, aggression, and sex, there has been relatively little work on the interactions between such systems. Yet it is obvious that an animal's various needs have to be delicately counterbalanced, and that there is a problem for nature in weighing one against the other. We may call this the trade-off problem.

In considering what an animal ought to do in a given situation, we have to remember that the various possible activities have different consequences, and most have both costs and benefits associated with them. Let us take, as an example, the courtship behaviour of the smooth newt (*Triturus vulgaris*). This has been the subject of intensive study by members of the Oxford Animal Behaviour Research Group, supported by the Science Research Council, and aided by various university colleagues.

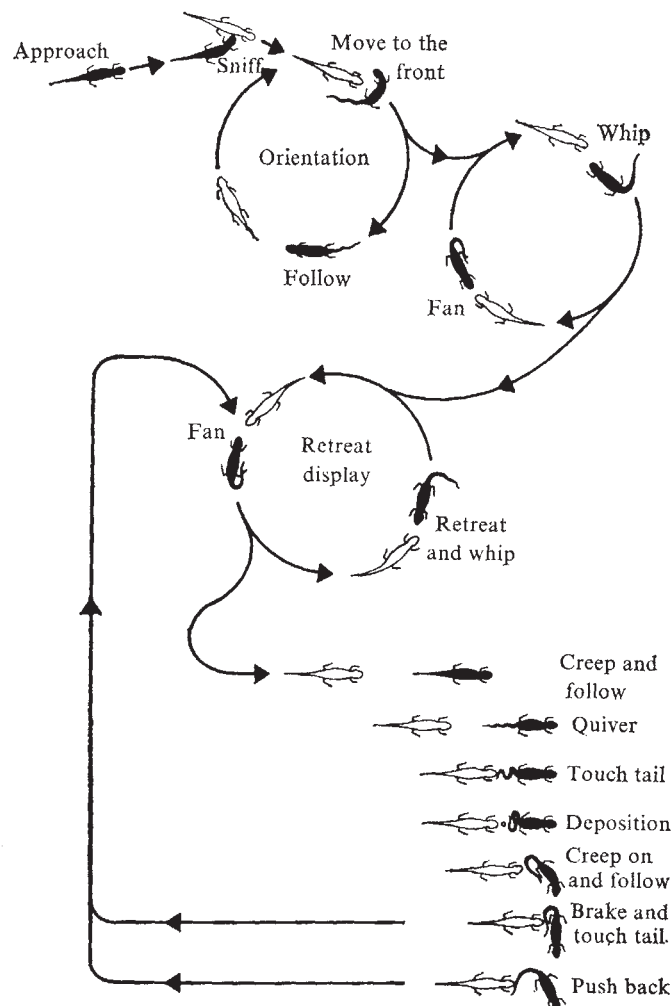
The courtship takes place on the bottom of a pond and is frequently interrupted by ascent to the surface of the water to breathe air. Observations show that the male newt breathes less often during courtship than he does normally. After the male has been to the surface of the pond to breathe he may be unable to find the female again when he returns to the bottom. Clearly, there is a cost associated with breathing during courtship, and it is not surprising that the males make breathing trips infrequently. However, although newts that are prevented from coming to the surface can probably remain alive by means of the oxygen dissolved in the water, they would be forced to become quiescent, so that courtship would no longer be possible. Thus there is also a cost associated with postponing breathing. The courtship of the male (Fig. 1) is designed to induce the female to pick up the spermatophore, which he deposits on the substratum. The female can only do this successfully if she follows the male closely. For this reason the courtship should not proceed too quickly, and the most successful courtships tend to be prolonged affairs. Thus, although the breathing problem can be alleviated by speeding up the courtship, this may make successful fertilisation less likely.

Mathematical formulation

Having touched on some of the problems involved in decision making in animals, we can now tackle the question of how we should aim to account for such decision processes.

It is difficult to foresee the day when the physiological analysis of behavioural control mechanisms will have advanced far enough to make much contribution to the explanation of decision making. Even if one knew all about the physiological mechanisms underlying breathing and courtship in newts, one would still not be able to predict when a male would decide to breathe rather than court a female. The decision will depend on

Fig. 1 A summary of the courtship behaviour of the smooth newt *Triturus vulgaris*. The black newt is the male. (From Halliday¹⁹.)



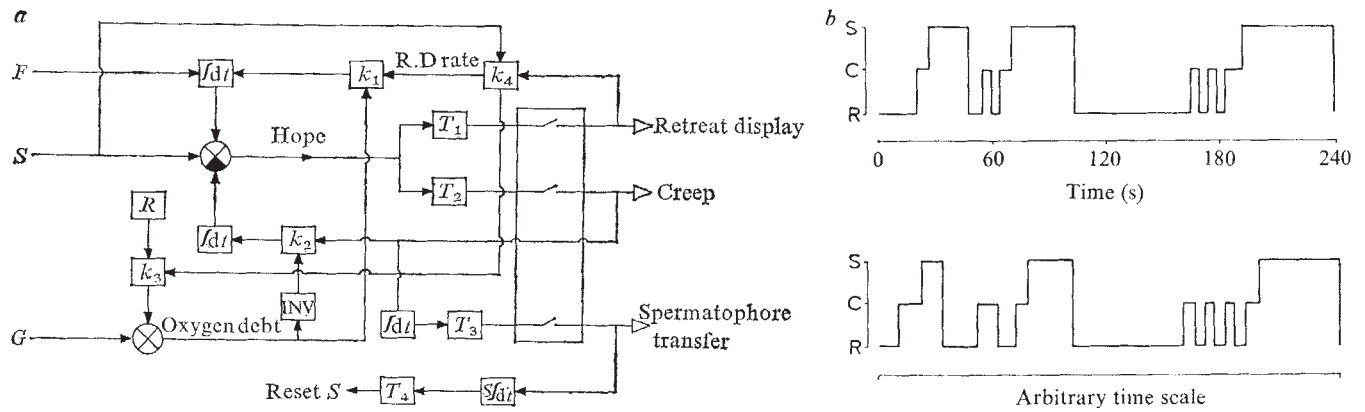


Fig. 2 a, A control model for the courtship of the male smooth newt. The following conventions have been observed: S , initial amount of sperm; F , initial sexual state of the female; G , initial value of oxygen debt; R , function representing the increase in oxygen debt with time; T_1 – T_4 , thresholds; k_1 – k_4 , modifiable parameters (modified from Houston *et al.*¹⁵); b, comparison of mean durations of various courtship activities of 22 newts (above) with that of the model (a). R, Retreat display; C, creep; S, spermatophore transfer phase (From Houston *et al.*¹⁵.)

how the animal balances the two tendencies against one another. We would expect the balance to be influenced by both proximal circumstances and ultimate ecological considerations. The difficulty is that physiological knowledge is essentially piecemeal, whereas the decision problem is holistic.

The traditional behavioural analysis of preference and choice is equally inadequate in accounting for decision making as a function of time. The main reasons for this are that such theory takes no account of the ecological relevance of the behaviour, and is often attempting to account for processes which are in principle unobservable from the viewpoint of the psychological experimenter⁸.

Clearly, as has long been recognised by economists, some kind of abstract mathematical formulation is required to handle situations of such complexity. The difficulty lies in relating mathematical formulae to physiological and behavioural reality. In recent years there has been considerable progress in this area, by physiologists and behavioural scientists, who have embraced various types of systems analysis. The approach adopted by our research group is to represent the motivational state of the animal by the vector x , envisaged as a point in a multidimensional space. The state is influenced both by events internal to the animal, and by the external stimuli perceived by the animal. The changes in motivation induced as a consequence of behaviour trace a trajectory in the motivational space⁷.

It is traditional to view the motivational state of an animal as driving the behaviour, or as the input to a control system of which behaviour is the output⁹. However, an alternative approach is to regard the behaviour as the input which controls the state of the system by virtue of its consequences. In other words, the animal is seen as controlling its own internal state through the medium of behaviour, in accordance with built-in optimal design principles. Optimal performance is attained by reference to an optimality criterion that is embodied in an objective function, which is a function of the state x and of the behaviour u , where u is a vector representing the current behaviour in a behaviour-repertoire space. The objective function incorporates the optimality criterion represented as a set of trade-off relationships between the state and behaviour of the animal and their associated costs and benefits. In other words it specifies the (supposed) net cost of being in a particular state, and net benefit of doing a particular behaviour pattern under a given set of circumstances. The objective function is envisaged as a property of the individual animal, presumably genetically determined, and possibly modifiable by learning. Thus objective functions are expected to differ from one individual to another, although they may be similar in related individuals.

We can imagine the animal's brain as controlling the state of its body. Since every behaviour u results in a change in bodily

state x , the situation is akin to that found in man-made process-control problems, where the state of the plant x is influenced by extrinsic factors, and by the controller u , which is designed to achieve some optimal condition of the plant. For this reason the equations, which relate the ever-changing consequences of behaviour to the resulting changes in x , are called the plant equations.

The optimal control problem is to deploy the behaviour u in such a way that a particular state function, generally called a Hamiltonian H , is maximised at every point along the resulting trajectory. In other words, the animal is designed to deploy its behavioural options in a manner which continuously maximises the Hamiltonian

$$H = \sum_{i=1}^n \frac{\delta K}{\delta x_i} x_i - K = \text{plant equations} - \text{objective function}$$

The argument underlying this formulation is derived from decision theory, in which, as outlined above, the essential ingredients are the assumption of a maximising principle, and the formulation of an hypothesis relating to the objective function. This hypothesis can be tested by direct observation of the animal. As an example, let us return to the courtship of the male smooth newt (*Triturus vulgaris*). On the basis of a number of elegant experiments by Halliday¹⁰⁻¹², a deterministic control model was formulated by Houston *et al.*¹³, which accurately simulates the timing of the courtship sequence (Fig. 2). This computer model provides the information required to derive the Hamiltonian equations, which will predict the behaviour on the basis of its associated costs and benefits. The next task is to formulate the plant equations and find an objective function which satisfies the Hamiltonian formulation. On the assumption that the courtship of the male newt is designed to maximise the probability of successful fertilisation of the female, Dempster¹⁴ proposed the formulation illustrated in Scheme 1.

A prime feature of the courtship is that it takes place under water, and this means that the oxygen supply of the male is a most important factor. Indeed, the causal model (Fig. 2) shows that oxygen debt is the variable primarily responsible for modulating the temporal organisation of the courtship sequence, and this has been confirmed empirically¹¹. The importance of oxygen supply is reflected in the plant equation (Fig. 3) which specifies only one state variable: oxygen debt x . Because the male can replenish his oxygen supply only by terminating the

Scheme 1 Simplified version of Dempster's¹⁶ formulation of the temporal organisation of the courtship of the male smooth newt, in terms of optimality theory.

Objective: Maximise likelihood of successful fertilisation

$$\int_0^{\infty} \left\{ \exp \left[-\Phi S(t)t \right] S(t)u_1(t)x(t) + S(t)u_3(t)x(t) \right\} dt$$

Plant equation:

$$\dot{x}(t) = -x(t)^{-\rho}u_2(t) + \theta$$

Work during courtship:

$$\frac{u_1(t)}{\delta} + \frac{u_2(t)}{\gamma} + \frac{u_3(t)}{\tau} \leq 1$$

Energy expended during courtship:

$$\int_0^{\infty} \left[\frac{u_1(t)}{\delta} + \frac{u_2(t)}{\gamma} + \frac{u_3(t)}{\tau} \right] dt \leq E$$

Hamiltonian:

$$H = e^{-\Phi S(t)t} S(t)u_1(t)x(t) + S(t)u_3(t)x(t) - F(t) \left[-x(t)^{-\rho}u_2(t) + \theta \right]$$

(Costate) Hamiltonian equation:

$$\dot{F}(t) = \frac{\partial H}{\partial x} = e^{-\Phi S(t)t} S(t)u_2(t) - \rho \frac{F(t)}{x(t)^{1+\rho}} u_2(t) + S(t)u_3(t)$$

states: S = spermatophore count (0–3)

controls: x = oxygen 'debt'

u_1 = rate of display behaviour (maximum rate δ)

u_2 = rate of creep behaviour (maximum rate γ)

u_3 = rate of spermatophore transfer (maximum rate τ)

parameters: Φ = basic discount rate of display behaviour

ρ = control parameter for rate of decrease of x through skin respiration during creep behaviour

θ = rate of increase of oxygen debt with time (during display and transfer behaviour)

E = maximal energy expended in courtship

F = costate variable = marginal value of likelihood of success per unit of x

courtship and visiting the surface of the pond to breathe, there is effectively a fixed energy budget during courtship. Given that the courtship activities of the male have a "typical intensity"¹⁵, the fixed energy budget implies that there is a limited period of time available for effective courtship. During this time the male has to display to the female sufficiently to induce her to follow him closely during the spermatophore phase (Fig. 1), when it is essential that the female should correctly pick up the spermatophore, which is deposited by the male on the substratum. It is likely that coyness on the part of the females is a means of testing the suitability of the male as a genetic partner.

Early in the courtship there is a high probability that the female will depart from the scene to breathe, to feed, or to seek another male. As the male continues to display this probability decreases, and we can suppose that during the time available, R , this probability is given by $\Phi e^{-\Phi t}$. On this basis we can express the expected probability of fertilisation, given that the female is still involved in the courtship

$$E \int_0^R u_1(t) + e^{\Phi S(t)t} u_3(t) S(t)x(t) dt$$

This formulation implies that the importance of spermatophore transfer increases exponentially as the courtship proceeds.

The objective function (see box), therefore, takes account of the probability of the female departing, as well as the male's sperm supply S and oxygen debt x . The Hamiltonian, based upon this objective function and upon the plant equation specified below, gives rise to a Hamiltonian equation with a term for each of the three types of behaviour u_1 , u_2 , and u_3 . The optimal behaviour sequence is arrived at by the newt choosing to do that behaviour which has the larger term in the Hamiltonian equation. In doing this the newt is in effect choosing that activity which confers the greatest benefit in terms of maximising the probability of successful fertilisation.

This formulation has proved to be highly successful in predicting the timing of the courtship in a variety of conditions. It also makes some specific predictions about the physiology of the male newt. For example, during the vigorous display behaviour, the male's oxygen supply diminishes, but during creep behaviour the model requires that the oxygen supply increase to some extent. In fact, the creep behaviour is much less energetic than display, and it is entirely possible that oxygen supply can increase by means of skin respiration. This somewhat startling prediction implies that the male deploys the creep behaviour as a means of prolonging the courtship. Moreover, some such idea is necessary to explain the fact, illustrated in Fig. 2, that the male oscillates between creep and display more frequently in the later stages of the courtship session, when his oxygen supply is presumably lower. Of course the prediction that oxygen supply actually increases during creep behaviour should be put to the experimental test. It is reinforced, however, by the observation that this particular species of newt not only has a more prolonged courtship than related species, but also has a larger crest¹⁶, which probably enhances skin respiration.

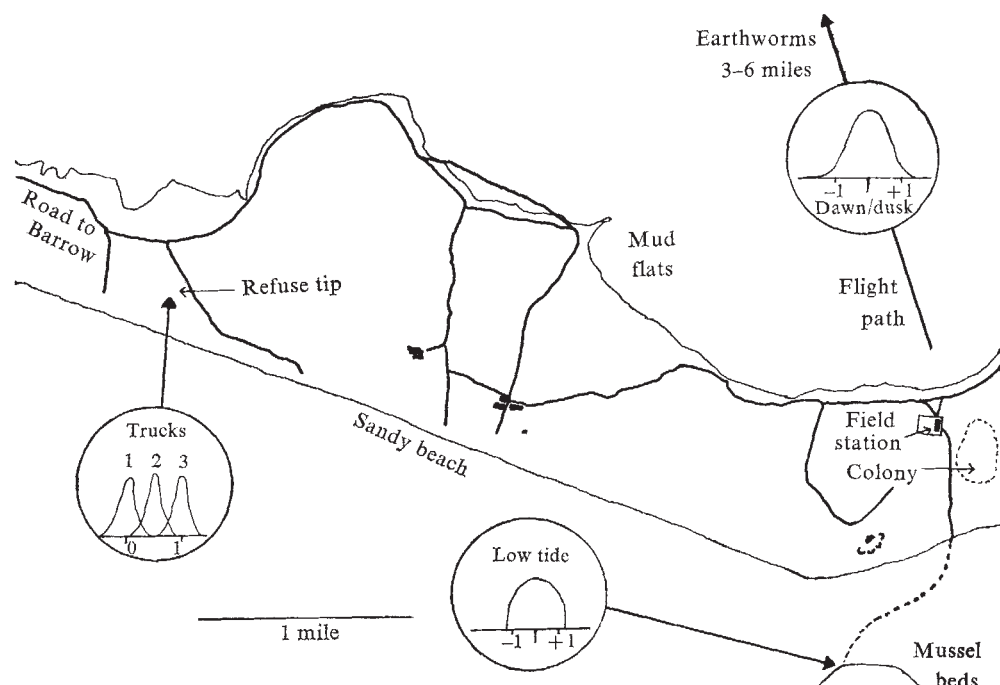
The reader may wonder why all this mathematical sophistication should be expended upon the courtship of newts. The answer is that so far newt courtship provides the most thoroughly worked test case of this general approach to animal decision making. This approach has widespread implications for theoretical biology, because it offers some insight into inverse optimality problems. Normal optimality problems pose the question of the best route to a goal, in particular circumstances. The inverse problem, which has been largely neglected by mathematicians, but is important for biologists, is that of what is being optimised by a particular structure, or a particular observed behaviour sequence. The general philosophy here is the following: if we know what a mechanism or system is designed to do, it will be much easier to find out how it works. In animal decision making it is essential to know that the decisions are about, before attempting to account for the mechanisms involved.

Natural selection

As biologists, we can expect natural selection to shape the decision-making processes of animals in such a way that the resultant behaviour sequences are optimally adapted to the current environmental circumstances. In other words, we can regard natural selection as a designing agent capable of producing the optimal design for any given set of conditions. Such considerations of optimal design apply just as much to the temporal organisation of behaviour as they do to the anatomical properties of animals. However, we cannot expect an individual animal always to behave optimally in its natural environment. Genetic variation between individuals, competition between individuals, the patchy nature of the environment, and changes in the nature of the environment, all combine to make it very unlikely that an individual animal can ever be perfectly adapted to its niche.

We can imagine, however, an 'ideal' animal that is perfectly adapted to its natural environment. The objective function of such an animal will be identical to a cost function, which is characteristic of the environment, in the sense that it reflects the selective pressures that moulded the objective function during the course of evolution. The cost function, therefore, specifies

Fig. 3 Map of part of Walney Island showing the positions of the most important feeding places in relation to the colony. Circles enclose graphs showing rough estimates of food availability.



the instantaneous level of risk incurred by (and reproductive benefit available to) an animal in a particular internal state, engaged in a particular activity in a particular environment. Such an ideal animal, perfectly adapted to its environment, will deploy its behavioural options so as to maximise the Hamiltonian, and thereby achieve that behaviour sequence which maximises its inclusive fitness in the prevailing circumstances.

In practice, we can distinguish two procedures by which optimal behaviour sequences can be investigated. In the first, a maximisation principle is assumed, and a particular form of the objective function postulated. This hypothesis is then tested in laboratory experiments. Ability to reproduce the behaviour of the animal is then taken as evidence in favour of the postulated objective function. This is the strategy used in the analysis of newt courtship outlined above.

In the second approach, an attempt is made to measure the components of the cost function empirically, by means of observations and experiments made in the field. The aim in this type of study is to determine whether animals in a particular natural environment are deploying their behavioural options in the optimal manner. The Hamiltonian formulation can then be used to generate behaviour sequences, or strategies, for a given environment, provided that sufficient is known about the cost function characteristic of the environment, and the plant equations characteristic of the animal.

As an example, let us consider the herring gull incubating its eggs, mentioned at the beginning of this article. This situation is being studied in detail by members of the Oxford Animal Behaviour Research Group, notably R. McCleery and R. Sibly, supported by the Natural Environment Research Council. The animals are studied intensively during the 4-week incubation period, particular attention being given to three main problems.

First, the factors influencing the internal state, of which the most important is hunger, and those affecting the animal's estimate of the environmental situation, of which the weather and food supply are of prime importance, are incorporated in the plant equations for the incubating bird. As part of the assessment of the animal's internal state, the whole nest including the sitting bird is weighed continuously by means of an electrically monitored balance buried in the ground. The measurements obtained in this way are capable of providing information about the loss of weight during fasting, and the gain of weight after foraging, with an accuracy of ± 20 g.

The gulls have a variety of possible feeding sites, at various distances from the nest, as indicated in Fig. 3. The availability of food fluctuates considerably in any one location, so that the bird has to choose among a continuously varying pattern of possibilities (Fig. 4)¹⁸. The availability of food as a function of time is computed from the tide cycle, the work routine of the men at the nearby refuse tip, the behaviour of earthworms in response to the weather, and so on. The extent to which the

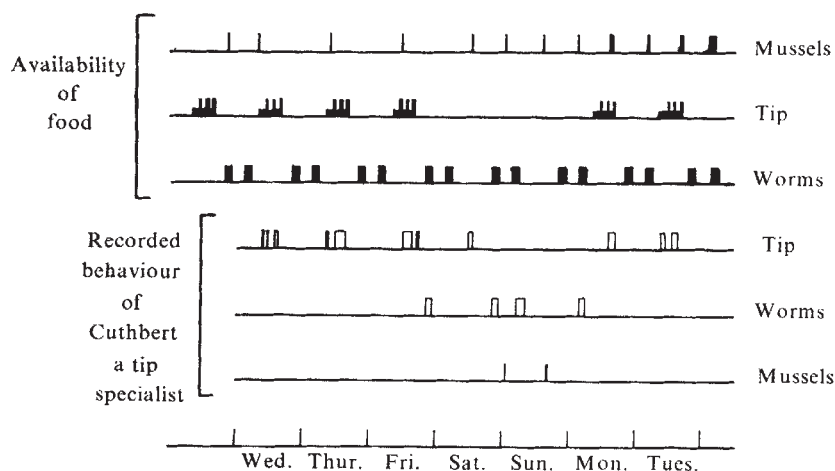


Fig. 4 Availability of food (above) during one week in the 1976 season, and (below) the foraging behaviour of one individual gull, record by radiotelemetry.

birds can exploit these opportunities depends on the willingness of the partner to take over the incubation duties, the probability of human interference near the feeding sites, and the vicissitudes of the weather. These variables are all monitored, either by direct observation, or by means of remote automatic monitoring devices. Weather variables, such as light intensity, wind speed, and so on, have a considerable influence on the foraging efficiency of the gulls, and must be an important factor in their foraging strategy. The returns that a gull can be expected to gain per unit time spent foraging are also estimated; by analysing samples of the food source, and checking this against regurgitations around the nest. All these factors taken together, when suitably calibrated and integrated with each other, can be used to obtain a good indication of the state of an individual bird in a given set of circumstances, and of the changes in state that are consequent on its behaviour.

Second, the behaviour of the individual birds on the territory can be monitored by direct observation from hides. Each bird is fitted with a radio transmitter so that it can be followed in a Land Rover when it leaves the territory, usually to forage for food. The birds are conspicuously marked so that they can be recognised amongst the thousands of others. Typically, a bird will fly from the territory directly to its chosen feeding ground. It may spend one or two hours feeding, often in competition with other gulls. It will then often bathe and drink in a fresh water puddle, before taking a 'rest' on a quiet part of the beach, or in a field. Such rest periods are generally communal affairs, although interactions between individuals are few. A considerable amount of time is spent preening and the question of whether gulls sleep on such occasions is currently being investigated. After a few hours away from the territory, the gull invariably returns home and takes over the task of incubation, relieving its partner.

The third problem is the cost function, which is a function of both the internal state of the animal, and of its behaviour. Obviously, for an incubating bird there is a risk attached to being in a particular state of hunger. It is not that the animal is likely to die while sitting on the nest, but rather that it may not find food very easily when it leaves the nest. If the animal had a reliable supply of food it could afford to risk a higher level of hunger than if its foraging fortunes were uncertain. In our study we attempt to measure such risks experimentally by removing one of an incubating pair of gulls, and studying the condition and behaviour of the remaining bird.

Similarly, when a bird leaves the nest as a result of disturbance or when pressed by hunger, there is a chance that its eggs will be eaten by a neighbour. The risk of egg predation can be measured by removing both of the nest owners and measuring the survival time of the eggs. Every aspect of the behaviour also has risks and benefits attached to it. Thus a bird foraging on the refuse tip may be poisoned, a gull on the shore line may get oil on its feathers. There is also the energetic cost of each activity to be taken into account. By systematically evaluating the cost and benefits involved in the behavioural routines of both members of mated pairs of gulls we aim to arrive at a mathematical formulation of the cost function.

In real life, an animal has to decide whether to sleep, feed, groom, and so on, at any particular time. In addition to its motivational state and the strengths of its various behavioural tendencies, the decisions reached will be heavily influenced by the decision criteria embodied in the objective function. If I believe that animal behaviour is subject to natural selection, I must conclude that the objective function is shaped by natural selection, because the decision criteria strongly influence the order in which animals go about their daily tasks.

The animal can be seen as carrying out a cost-benefit analysis in making its decisions. If the animal is not closely adapted to its environment, it may not make the analysis correctly; that is its objective function may not conform to the cost function characteristic of the current environment. Nevertheless, the animal is still an optimising agent with respect to its own objective function.

I do not intend to suggest that the individual animal necessarily carries out the cost-benefit analysis in a cognitive fashion, although this may occur to some extent in intelligent animals. The form and pattern of the behaviour is the outcome of a design operation which aims at the optimal compromise between the competing selective pressures characteristic of the environment. Obviously in a non-stationary environment, there will be an advantage in organising the decision-making processes in a flexible manner. Thus we can expect the laws governing learning to be designed in such a way that they tend to shape the objective function of the individual, and make it more like the cost function of the current environment. To study such phenomena we have to know something about the make-up of the individual animal, and something about its ability to adapt to the environment. This is where the herring gull study is so instructive. There is no question that individuals differ greatly. Some specialise in foraging for mussels, others for crabs, using quite different techniques. Some specialise in stealing eggs and chicks from neighbours, and others have apprenticed themselves at the local corporation refuse tip. Indeed, an important aspect of the optimal decision-making approach, outlined in this article, is that attention is concentrated on the behaviour of individuals, in contrast to the more traditional approaches to the study of animal behaviour, which rely on statistical inferences based upon the study of large numbers of animals. This attention to detail is made possible by the fact that optimality theory provides a way in which precise mathematical functions can be used within the normal scientific framework of hypothesis, prediction, and test. Hypothesised functions, tailor-made for the individual animal, can be used in optimality computations to predict sequences of behaviour that should occur in observed circumstances and conditions, and these predictions can be compared with the behaviour observed in the field or laboratory.

What then is likely to be achieved by the application of optimality theory to animal decision making, or by empirical studies designed to test predictions of optimality theory? First, the development of the theory itself has proved to be extremely instructive in forging links between ecology and behaviour¹⁸, between behaviour and economics¹⁹, and between environmental physiology and behaviour⁶. As I have mentioned elsewhere²⁰, we are entering a new phase of interpretation of animal behaviour, in which the complexity of animals is taken as a challenge, rather than as an excuse for mysticism or for sceptical over-simplification.

Second, we cannot expect to understand decision-making processes without also appreciating the selective pressures that shaped them. In many areas of biology it is helpful to understand the function of the material being studied, but in decision making it is essential, because decisions inevitably reflect the trade-off and compromise among the design features of the animal. Optimality theory provides a self-checking procedure, which is a considerable improvement on the traditional open-ended approach to the survival value of behaviour⁹.

Third, empirical studies ought to be part of any mathematical approach to biology, where there is a strong temptation towards arm-chair theorising. These studies should tell us, not merely whether the theory is right or wrong, since it is bound to be wrong in some respects, but also something about the mental competence of the animal themselves. Already we have seen that herring gulls adapt individually to their complex, ever shifting, circumstances. When we know more about how to account for the apparent decisions we observe and how good the animals are at matching their decisions to the real world, we may be in a position to investigate the mechanisms used by animals in adapting their time budgets to changing circumstances.

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articles

On the optical identifications of five X-ray sources

H. V. Bradt, K. M. V. Apparao*, G. W. Clark, R. Dower, R. Doxsey, D. R. Hearn, J. G. Jernigan, P. C. Joss, W. Mayer, J. McClintock & F. Walter†

Department of Physics and Center for Space Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

The data from a recently completed survey of the galactic plane with the SAS-3 modulation collimators provide precise (20"–60") celestial positions of galactic X-ray sources. Preliminary positions of 60" precision are reported for five sources. One of these led to the identification of the star, γ Cas, as an X-ray source, and the others lend substantial confidence to previously proposed optical identifications: 3U0352+30 = X Per, 3U1145–61 = HEN 715, GX301–2 = WRA977, and GX304–1 = MMV star. These identifications seem to establish the existence of a previously suggested class of De-star X-ray emitters.

THE SAS-3 X-ray astronomy observatory carries rotation modulation collimators (RMC) with which the positions of X-ray sources in the energy range 2–11 keV can be measured^{1,2} with a precision of 20" for the brighter sources³. In uncrowded regions, the detection threshold of a typical observation (1–2 d) is about 3 μ Jy (see footnote to Table 1). A programme of observations of the entire galactic plane ($|b^{\text{II}}| \lesssim 10^\circ$) has now been completed and the analysis is in progress.

The objective of this programme is the identification of radio, infrared and optical counterparts of the X-ray sources. At present there are about 10 well established identifications of stellar optical objects with hard ($\gtrsim 2$ keV) X-ray sources. Most of these identifications are compelling because of positional coincidences coupled with coincident temporal phenomena such as identical orbital periods at both optical and X-ray wavelengths. A comparable number of additional identifications with varying degrees of confidence have also been proposed.

We describe here the survey of the galactic plane and present the results of five position measurements derived from it. One of these led to the identification of γ Cas as an X-ray source, and the others substantially increase our confidence in previously proposed identifications. These results are derived from the quick-look data and an early version of the analysis system which limits the positional precision to 1.0' (90% confidence). They have been announced earlier in preliminary forms^{5–8}.

Other early SAS-3 RMC positions have been reported for the

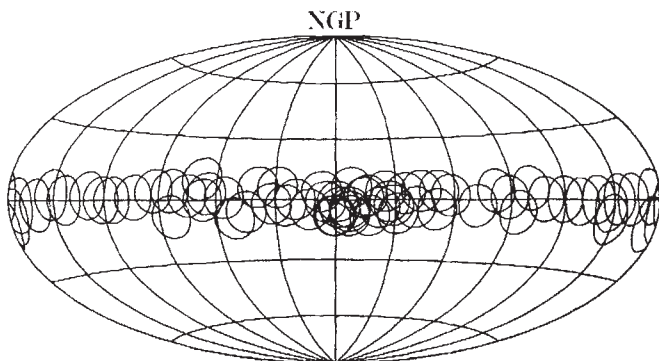
following galactic X-ray sources: Aql X-1, Ser X-1, GX9+9, and GX17+2 (ref. 9); 3U0143+61 and 3U1956+11 (ref. 10); Algol (ref. 2); MX1553–54 (ref. 11); the transients MX1803–24 (ref. 12), A1524–61 (ref. 13), and A0620–00 (ref. 1); the steady counterparts to two burst sources, 3U1733–27 (ref. 14) and A1905+00 (ref. 15); and the mid-galactic latitude source, 3U0044+32 (ref. 16). These and the positions reported herein constitute the first SAS-3 list ('1S') of galactic source positions. Subsequent papers will present '2S' positions as they become available. The latter will be derived from larger samples of data analysed with an improved system which yields the higher precision quoted above.

The galactic survey

The two SAS-3 modulation collimators have periodic triangular response functions of 2.3' and 4.5' FWHM respectively and $12^\circ \times 12^\circ$ FWHM fields of view. The celestial locations of the fields of view for the survey observations (Fig. 1) were chosen to yield two to three independent measurements of most sources and to provide substantial exposure to galactic latitudes $\pm 12^\circ$ in the region $-60^\circ < \mathcal{L}^{\text{II}} < +40^\circ$ and $\pm 8^\circ$ elsewhere. The durations of the observations ranged from 0.5 to 2 d. Simultaneous X-ray data and optical aspect data which can be analysed for X-ray positions were obtained for about 30% of this time. Thus the useful duration of each observation ranged from $\sim 1 \times 10^4$ s to $\sim 5 \times 10^4$ s.

The celestial positions of the X-ray sources are derived from a cross-correlation analysis¹⁷ and a fast mapping algorithm. The

Fig. 1 Fields of view of the SAS-3 rotating modulation collimator during the survey of the galactic plane in galactic coordinates. The galactic centre is at the centre of the figure and the north galactic pole (NGP) is at the top.



*Permanent address: Tata Institute of Fundamental Research, Bombay, India.

†Present address: Department of Astronomy, University of California, Berkeley, California 94720.

Table 1 X-ray sources

SAS-3 designation	Other designations*	α	Position (1950) δ	ℓ^{II} b^{II}	Error radius ($\sim 90\%$)	Flux density*† (2–6 keV)	X-ray* periods	Luminosity‡ (2–6 keV)
IS0053 + 604 (γ Cas)	MX0053 + 60 MX0049 + 59(?)	00 h 53 min 41.3 s 13.4221°	60°26' 57" 60.4492°	123.6° –2.1°	1.0'	20 μ Jy		2×10^{33} erg s $^{-1}$
IS0352 + 308 (X Per)	3U0352 + 30	03 h 52 min 14.4 s 58.0600°	30°53' 42" 30.8950°	163.1° –17.1°	1.0'	35 μ Jy	13.9 min 22 h? 581 d?	5×10^{33} erg s $^{-1}$
IS1145 – 619 (HEN 715)	3U1145 – 61	11 h 45 min 30.6 s 176.3775°	–61°55' 40" –61.9278°	295.6° –0.2°	1.0'	120 μ Jy		3×10^{35} erg s $^{-1}$
IS1223 – 624 (WRA 977)	GX301 – 2 3U1223 – 62	12 h 23 min 50.2 s 185.9592°	–62°29' 35" –62.4931°	300.1° –0.0°	1.0'	50 μ Jy (< 10 –200; 25 keV)	11.6 min	4×10^{36} erg s $^{-1}$ (2–30 keV)
IS1258 – 613 (MMV star)	GX304 – 1 3U1258 – 61	12 h 58 min 14.3 s 194.5596°	–61°20' 01" –61.3336°	304.1° 1.2°	1.0'	80 μ Jy (< 8 –60; 25 keV)	4.5 min	

*See text for refs.

† μ Jy $\equiv 1.000 \times 10^{-29}$ erg s $^{-1}$ cm $^{-2}$ Hz $^{-1}$
 $\equiv 2.418 \times 10^{-12}$ erg s $^{-1}$ cm $^{-2}$ keV $^{-1}$
 $\equiv 1.509 \times 10^{-3}$ keV s $^{-1}$ cm $^{-2}$ keV $^{-1}$ For a power-law spectrum of slope $\alpha = 1.08$ (for example, the Crab nebula*):1.0 μ Jy at 3.6 keV corresponds to an integrated flux of 0.96×10^{-11} erg s $^{-1}$ cm $^{-2}$ (2–6 keV) or ~ 0.6 Uhuru counts.1.0 μ Jy at 5.2 keV corresponds to 2.2×10^{-11} erg s $^{-1}$ cm $^{-2}$ (2–11 keV). I_{Crab} (3.6 keV) = 1,580 μ Jy; I_{Crab} (5.2 keV) = 1,060 μ Jy.

‡For the distances given in Table 2.

minimum intensity (counts cm $^{-2}$ s $^{-1}$) which is detectable in these cross-correlation 'maps' at the m standard deviation level can be shown to be $I_{\text{min}} = (m\pi^2/A) (B/8T)^{1/2}$ where B is the background rate (counts s $^{-1}$), A is the effective area at the peak of the response function (cm 2), and T is the effective observation time. An isolated source near the centre of the coarse field of view will be detected at the 7σ level ($m = 7$) in one of the two collimators ($A = 180$ cm 2 , $B = 30$ s $^{-1}$ and $T = 25,000$ s) if its intensity is

$$I_{\text{min}} = 4.7 \times 10^{-3} \text{ counts cm}^{-2} \text{ s}^{-1} \text{ (2–11 keV)}$$

$$\approx 2.3 \mu\text{Jy (2–11 keV)}$$

An intense source in the field of view will raise the effective background for the detection of a weak source. In the presence of a point-like source with the intensity of the Crab nebula at the centre of the field of view (380 counts s $^{-1}$, 2–11 keV), we have $\langle B \rangle = 30 + 380/2 = 220$ counts s $^{-1}$ and obtain $I_{\text{min}} = 6 \mu\text{Jy} (7\sigma)$. Furthermore, the bright source will exhibit a ring pattern which decreases in amplitude with radius from the source^{17,18}. This pattern can mask a nearby weak source, and therefore must be subtracted from the map¹⁷. This can be done with an uncertainty of $< 5\%$ of the bright source intensity. In our example, the residual rings from the 2.3' collimator, after subtraction of the Crab-like source, would have an amplitude of 5 μ Jy 1° from the source and 2 μ Jy at 5°.

Results

The position measurements reported here are summarised in Table 1. Characteristics of the optical candidates are summarised in Table 2 and finding charts are shown in Fig. 2. Of the five X-ray sources discussed here, one was identified in the course of this work (IS0053 + 604/ γ Cas), two were previously known and had convincing and well-studied optical counterparts (3U0352 + 30/X Per and GX301 – 2/WRA 977), and two have suggested counterparts which become credible with the present refined positions (3U1145 – 61/HEN 715 and GX304 – 1/MMV star).

Each of the positions was derived from at least four orbits of data and from both modulation collimators. Systematic errors in the positions dominate the statistical errors. We find the former to be no more than 30" to 40" based on observations of identified sources. Thus we report 60" error radii in this work as a conservative ($> 90\%$ confidence) estimate before the final analysis of the data. For each source, the multiple positions derived from individual orbits and collimators all lie well within this error circle.

The celestial areas defined by these circles are smaller by factors of 3–25 than the error regions obtained previously with the Uhuru¹⁹ and the Copernicus²⁰ satellites (see Fig. 2). In the case of IS0053 + 604, there are no other position determinations of comparable precision. The proposed optical counterpart of each of the five sources lies within the SAS-3 error circle. The small error regions and the unusual character of these stars lend substantial confidence to the proposed identifications.

Table 2 Optical counterparts*

Star	Apparent magnitude	M_V	Type	Optical periods	Estimated distance	Angular distance from X-ray position
γ Cas (IS0053 + 604)	$V \sim 1.6$ –3.0	~ -4.5	B0.5(II–V)e	0.7 d?	0.30 kpc	12"
X Per (3U0352 + 30)	$V \sim 6.0$ –6.7	~ -3.6	O9.5(III–V)e	13.9 min? 584?	0.35 kpc	22"
HEN 715 (3U1145 – 61)	$V = 9.0$	~ -3.5	B1Vne		1.5 kpc	21"
WRA 977 (GX301 – 2)	$V = 10.8$	~ -6	B1.5Ia	23 d 11.6 min?	2.0 kpc	6"
MMV star (GX304 – 1)	$V = 14.7$					27"

*See text for refs

The five sources

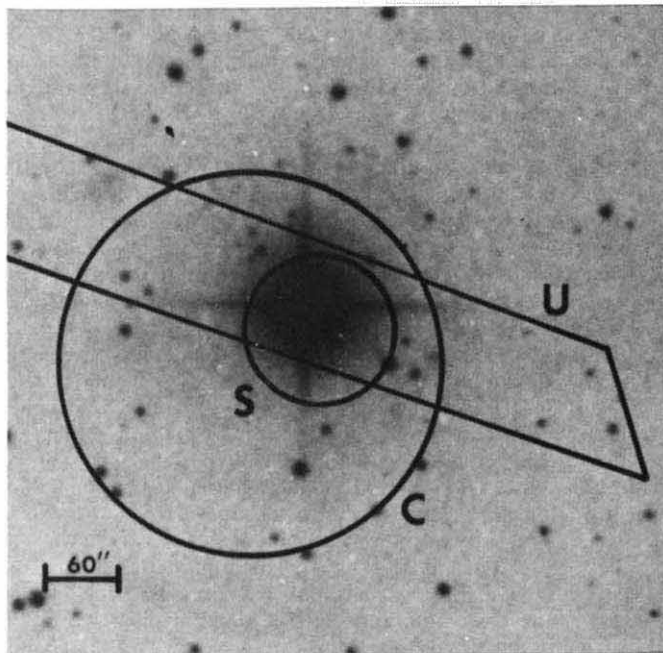
X-ray emission from **IS0053+604/γ Cas** was detected by SAS-3 during 30–31 December 1975 with both modulation collimators and the source was designated MX0053+60 (ref. 5). This source was the highest point ($\sim 15 \mu\text{Jy}$, 2–11 keV) in the $10^\circ \times 10^\circ$ field of the cross-correlation maps which were constructed from seven orbits of data (1.3×10^4 s). For Table 1, we assume the spectral shape of the Crab nebula and quote a 2–6 keV flux density of

$20 \mu\text{Jy}$. The X-ray position lies $10''$ from γ Cas which we identify as the optical counterpart.

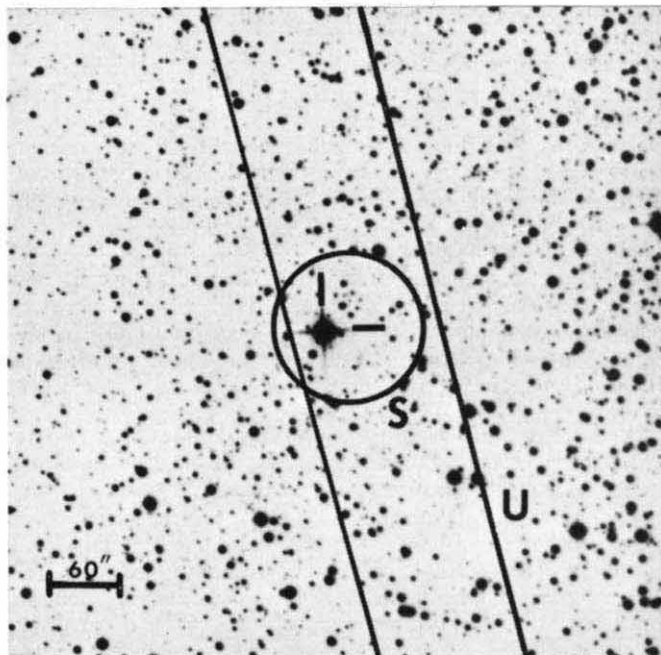
An X-ray source, MX0049+59, was detected earlier with OSO-7, 1.3° or about three standard deviations from γ Cas (ref. 21). Independent X-ray observations of this region in December 1975 and January 1976 with the Copernicus satellite²² yielded a positive signal from the region of γ Cas with a 3° uncertainty in celestial position. The source intensities derived from these several

Fig. 2 The X-ray error regions (U = Uhuru, C = Copernicus, S = SAS-3) and the proposed optical counterparts for four of the five sources. X Per is the very bright star in the field; the other candidates are marked. The photograph for the X Per region is from the blue Palomar Sky Survey plate (National Geographic Society); for HEN 715 and WRA 977, from the ESO quick blue survey; for the Mason, Murdin, and Visvanathan (MMV) star⁵⁴, from a plate taken by Canizares and Hiltner with the 4-m telescope (V filter) at CTIO.

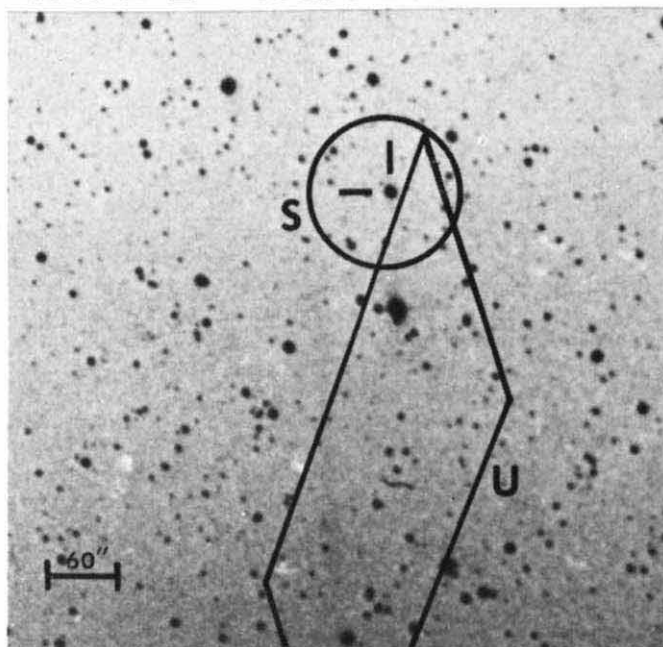
3U0352+30 / X Per



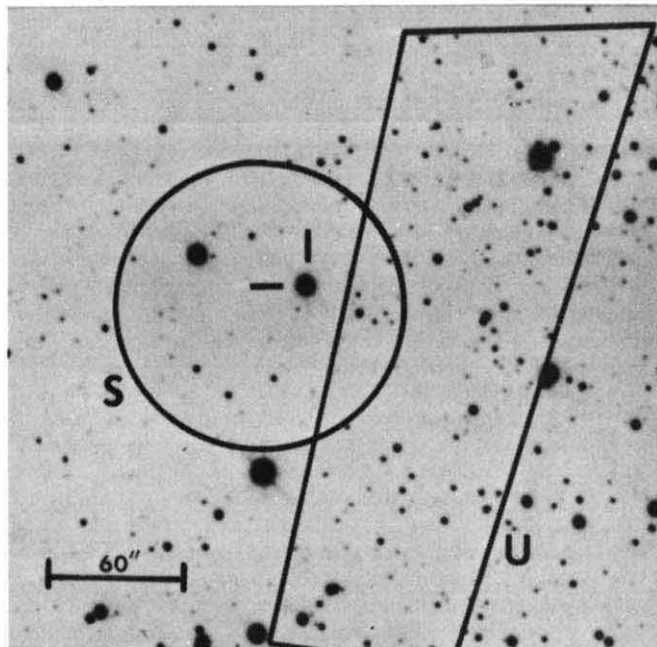
3U1145-61 / HEN 715



GX301-2 / WRA 977



GX304-1 / MMV star



observations are comparable, within about 50%. This supports the view that γ Cas is the only source in this region. The quoted intensities and the absence of an Uhuru sighting¹⁹ suggest that the intensity is variable.

γ Cas is a B0.5 (II–V)e star with variable intensity ($V = 1.6$ – 3.0), variable line profiles, $M_V \sim -4.5$, and distance ~ 300 pc (see summaries in refs 23, 24). The spectral features indicate that the star is rotating rapidly, and variations of the emission line profiles suggest a 0.7-d period²⁵. It is an exceptionally variable Be star and in this respect is similar to X Per (ref. 23).

At the distance of 300 pc the 2–6 keV X-ray luminosity is $\sim 2 \times 10^{33}$ erg s⁻¹. A search for 44 Å emission by SAS-3 yielded an upper limit of ~ 130 μ Jy ($\sim 1/50$ the Cygnus Loop flux) in the energy ranges 0.10–0.28 keV and 0.4–0.8 keV. In the absence of a substantial interstellar cut off at ~ 0.6 keV, this limit and the 2–11 keV flux indicate a temperature $kT \gtrsim 1.5$ keV for a thermal spectrum, or a spectral index $\alpha \lesssim 1.0$ for a power law energy spectrum.

X rays from the 3U0352+30/X Per system were first detected with Uhuru at a 2–6 keV flux density of 35 μ Jy (ref. 19) and later with Ariel V at a comparable level²⁶. A 13.9-min X-ray period with peak-to-peak amplitude of 40% has been discovered²⁷. Also possible 22-h (or 11-h, ref. 27) and 581-d (ref. 28) periods have been reported. The X-ray source also exhibits irregular variability on time scales of minutes²⁹.

X Per, by far the brightest star in an early Uhuru error box, was proposed as the optical counterpart by several groups^{30–32}. A $V = 12$ star, ADS 2859B, which lies 22.5" from X Per has been suggested as the counterpart²³. The present results cannot exclude it. Its colours and spectrum indicate, however, that it is a normal M III star with no peculiarities which would suggest that it is the X-ray source³³.

X Per is rotating rapidly (~ 400 km s⁻¹, ref. 32) and is classified²³ as a 09.5 (III–V)e star with $M_V \sim -3.6$. Its intensity varies slowly on time scales of months or years from $V = 6.0$ to $V = 6.7$ (refs. 34, 29). Spectral studies yield evidence for a 584-d period³⁵, and narrow band photometry at 4,686 Å provides an indication of episodes of 13.9 min variability (ref. 36 and Canizares, personal communication). Searches for this episodic phenomenon on other occasions and by other observers have not yet confirmed it³⁷. Brucato and Kristian³² place X Per in the Perseus 2 association at a distance of 350 pc, which implies an X-ray luminosity of 5×10^{33} erg s⁻¹.

The 3U1145–61/HEN 715 system has a maximum X-ray flux density of 120 μ Jy (2–6 keV) which is variable by a factor of 5 (ref. 19). The B-emission star HEN 715 (SAO 251595) has been suggested previously as a candidate on the basis of the Uhuru position^{38–40}. It is relatively bright ($V = 8.95$) and blue ($B - V = 0.2$, $U - B = -0.85$) and exhibits fluctuations in brightness ($\Delta B \approx 0.02$, ref. 39). Its spectral classification is B1Vne (ref. 41). We estimate the distance of this star to be 1.5 kpc if its luminosity is that of a B1 main-sequence star. This leads to a maximum luminosity of 3×10^{35} erg s⁻¹.

The GX301–2/WRA 977 system was first detected by Lewin *et al.*⁴² and McClintock *et al.*⁴³ during balloon observations which showed the source to be bright and variable in intensity by a factor of > 20 at high X-ray energies (< 10 – 200 μ Jy, 18–36 keV, ref. 44). At 2–6 keV, it is relatively weak (50 μ Jy) and variable by a factor of 3 (ref. 19). More recently, it has been found to exhibit an X-ray periodicity of 11.6 min (ref. 45) and to feature a strong iron absorption edge in its spectrum⁴⁶.

The emission line star WRA 977 ($V = 10.84$) has been proposed by Vidal⁴⁷ and Jones *et al.*³⁹ to be the optical counterpart. The star is heavily reddened ($E_{B-V} = 1.8$). The B1.5Ia classification suggests an absolute magnitude $M_V = -6$ and a distance of 2 kpc (ref. 47). This yields a 2–30-keV maximum X-ray luminosity of $\sim 4 \times 10^{36}$ erg s⁻¹.

At optical wavelengths, a tidal distortion light curve indicates a binary period of 23 ± 1 d (ref. 48). This has been confirmed by Petro (personal communication) who analysed a 3-yr compilation of photometric data provided by many observers. Finally, Mauder⁴⁹ reports that photometric data provide weak evidence

for the 11.6-min X-ray period. Thus, the established optical binary period of 23 d in WRA 977 has not been detected at X-ray wavelengths and the 11.6-min X-ray pulse period in GX301–2 has not been detected convincingly at optical wavelengths. It is our view, however, that the SAS-3 position removes any residual doubt about the identification of WRA 977 with GX301–2.

The GX304–1/MMV star system was first detected at X-ray wavelengths from balloons by Lewin *et al.*^{50,51}. It is another of the small class of X-ray sources which have been detected at energies up to ~ 50 keV. Like GX301–2, it is highly variable in intensity at all energies. At 2–6 keV, its flux density is 80 μ Jy with a variability factor of 5 (ref. 19) and at 20–40 keV the factor is more than 7 (< 8 – 60 μ Jy, refs 43, 44). It has recently been found to be a slow X-ray pulsar with a period of 4.5 min (refs 52, 53).

The celestial region of this source is shown on a deep plate (Fig. 2) taken by Canizares and Hiltner at CT10 with the 4-m telescope. The marked star ($V = 14.72$ and $B - V = 1.80$) has been proposed by Mason, Murdin, and Visvanathan (MMV) as the optical counterpart⁵⁴. They report that this star exhibits H α emission with a FWHM of 700 km s⁻¹ and an equivalent width of 12 Å and that the absorption spectrum suggests a spectral type between B2 and A0. We do not attempt to estimate the distance to the star because it lies in a heavily obscured region of the sky (the Coalsack). It is unlikely that the bright star 1.5' south of this star is the counterpart as proposed earlier by Huckle *et al.*⁵³. It lies outside the conservative SAS-3 error circle and its colours do not suggest an early-type star (W.A. Hiltner, personal communication).

Discussion

These results seem to establish the existence of the class of accreting binary X-ray sources, suggested by Maraschi, Treves, and van den Heuvel⁴⁰, wherein the source of accreting material is a rapidly rotating main-sequence Be star. These authors propose that such stars with masses as low as 8–10 $M_\odot$ could become X-ray sources through the accretion of circumstellar material on to a companion neutron star. They argue that the existence of such binary systems is reasonable from an evolutionary point of view.

The γ Cas, X Per and HEN 715 systems apparently belong to this class of X-ray emitters. The optical members are emission line, 09.5-B1, stars with luminosity classifications which are consistent with main-sequence membership. Two of these, γ Cas and X Per, are known to be rapid rotators^{25,32}, with angular velocities that may be sufficient to yield approximately zero effective gravity in the equatorial regions^{25,40}.

The spinup rate of the 13.9-min X-ray pulsar in the X Per system indicates that the compact accreting companion is a neutron star and not a white dwarf²⁸, although the latter would be consistent with the low X-ray luminosity^{55,56}. The similarity between the X Per and γ Cas systems in X-ray luminosity, absolute magnitude, and in their unusually large optical variability²³ suggests that γ Cas also has a compact companion undergoing accretion. The nature of the compact member would be clarified if the system is found to exhibit periodic X-ray pulsations. Optical studies of these two stars do not exclude the existence of low-mass ($< 2M_\odot$) secondaries^{24,57}.

The data indicate that this type of system can give rise to an unusually low luminosity ($L_x \sim 10^{33}$ – 10^{34} erg s⁻¹) compared with that of other hard (> 2 keV) galactic X-ray sources (10^{35} – 10^{39} erg s⁻¹). The only other known example of such a low luminosity is Algol (ref. 2) with $L_x \sim 1.6 \times 10^{31}$ erg s⁻¹. This radio flaring object^{58,59} is probably a very different type of system⁶⁰.

In contrast to the Be main-sequence stars, WRA 977 is a supergiant. Its X-ray partner is a slow pulsator with a luminosity which is quite high ($\sim 4 \times 10^{36}$ erg s⁻¹) and variable. This system is similar to the well known pulsating X-ray binary systems, 3U0900–40 and SMC X-1. It is well established that the X-ray emission from these objects arises from accretion on to a rotating magnetised neutron star (see refs 61, 62). Both a stellar wind and overflow of material from a critical potential lobe may be involved^{63,64}. The extremely hard and variable X-ray spectrum of GX301–2 is unusual even among these sources^{65,66}.

The X-ray source GX304–1 is similar to GX301–2 in that it has

a hard spectrum, a variable intensity at all energies and is also a slow X-ray pulsar. The tentative classification of its optical partner as B2 to A0 with a broadened H α emission line^{5,4} suggests, however, that this might be another example of the Be-star systems discussed above.

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Generation of Icelandic rhyolites by melting of plagiogranites in the oceanic layer

Haraldur Sigurdsson

Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island 02881

Trondhjemite and quartz diorite xenoliths ejected in recent Icelandic eruptions are believed to be derived from plagiogranites within layer 3, formed by fractional crystallisation of basaltic magmas in the lower crust. Evidence indicates that rift jumping to older crust can lead to partial melting of plagiogranites at depth and their eruption as rhyolitic magmas.

THE volume of rhyolites exposed within the lava pile in Iceland is disproportionately large (10%), with individual rhyolite complexes covering areas up to 450 km² (refs 1, 2). The pronounced bimodal compositional distribution of basic and acid extrusives (Fig. 1) suggests, furthermore, that a process of shallow-level fractional crystallisation in subvolcanic magma chambers cannot alone account for the origin of these acid magmas. The ⁸⁷Sr/⁸⁶Sr and Pb-isotopic evidence³⁻⁵, however, precludes the possibility of generation of the Icelandic rhyolites by fusion of old continental crustal material at depth. An alternative model has recently been proposed⁶⁻¹⁰ to account for the origin of the Icelandic rhyolites by hydrous partial melting of basalts within the crust. This hypothesis is inspired by the experimental work of Kushiro^{11,12} on the synthetic system Di-Fo-SiO₂-H₂O at 20 kbar, where a silica-rich liquid was generated at 960 °C. There are three principal obstacles to deriving Icelandic rhyolites by hydrous anatexis of basalts within the crust.

(1) The process dictates that the resulting acid melts be H₂O saturated, contrary to the evidence of Icelandic rhyolites which are often extruded as dry obsidian lavas or solidify at shallow levels as pyroxene-bearing granophyres, lacking hydrous phases. Many rhyolitic eruptions in Iceland are, of course, violently explosive events, clearly associated with a water-rich vapour phase. The oxygen isotope evidence is consistent with incorporation of some low-¹⁸O meteoric water into these magmas. Oxygen-isotope geochemistry of the acid rocks^{10,13} shows that the tephra products of acid explosive eruptions have $\delta^{18}\text{O}$ in the 0.4-2.5‰ range, whereas obsidian and rhyolite lavas have $\delta^{18}\text{O}$ values of 4-6 ‰. Thus the data are in agreement with the idea that the water-propellant of acid explosive eruptions in Iceland is largely meteoric water which has diffused into acid magma in subvolcanic magma chambers¹⁴.

(2) Anatexis of basalt in the presence of H₂O at pressures comparable with the lower Icelandic crust (5 kbar) generates andesitic and dacitic melts analogous to the calc-alkaline suite of island-arcs¹⁵ and unlike Icelandic rhyolites (Fig. 2).

(3) No field evidence has been presented so far for the existence of amphibolites or other suitable metabasite starting material under Iceland, nor have xenoliths of possible refractory phases of this process been erupted.

An alternative model proposed here for the origin of Icelandic rhyolites is the melting of plagiogranites within the oceanic layer. By analogy with ophiolite complexes¹⁷⁻²⁴

and petrological models of the oceanic crust^{20,21,25}, it is proposed that layer 3 under Iceland is composed of layered (cumulate) mafic rocks at the base, overlain by gabbros which grade upwards into leucocratic differentiates: trondhjemites and quartz diorites. This model of the oceanic layer under Iceland is supported by the ejection of plagiogranite xenoliths in several recent eruptions and the occurrence of plagiogranites in ophiolite sequences. The model is also consistent with the geochemical evidence, such as the bimodal compositional distribution of Icelandic volcanics and the dry character of the rhyolites.

Icelandic plagiogranite xenoliths

Fragments of leucocratic rocks have been ejected in a number of recent eruptions in Iceland, notably the 1963–67 eruption of Surtsey and the 1875 plinian eruption of Askja. Most finds are in basaltic scoria cones in the eastern and Snaefellsnes zones, that is, outside the western volcanic zone²⁶. Most Surtsey xenoliths have undergone extensive partial fusion and assimilation of the acid liquid into the basaltic melt, leaving a refractory residue of anorthitic plagioclase, tridymite and more rarely cordierite and mulite. Study of leucocratic xenoliths from Surtsey has shown

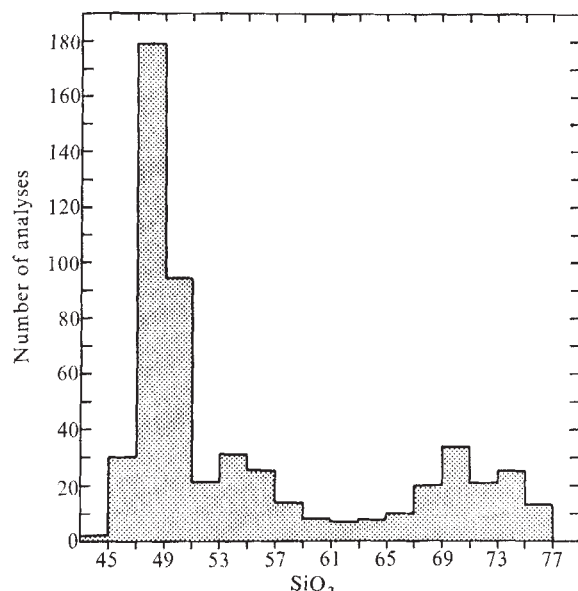


Fig. 1 Bimodal distribution of SiO_2 in 542 chemical analyses of volcanic rocks from Iceland.

that a complete gradation exists between anorthite-tridymite refractory assemblages and xenoliths of trondhjemite and quartz diorite relatively unaffected by fusion²⁶. The texture of Icelandic plagiogranites is distinct from that of granophyres in Tertiary and Quaternary intrusive complexes exposed in eastern and western Iceland. The majority of the plagiogranite xenoliths contain 55–85% plagioclase, 10–35% quartz, minor clinopyroxene, K-feldspar, accessory zircon and variable amounts of intergranular pale brown to colourless glass. Plagioclase ranges from An_{20} to An_{44} and typical plagioclase composition in Surtsey xenoliths is $\text{An}_{35}\text{Ab}_{64}\text{Or}_1$. Plagioclase in Askja xenoliths is comparable but more potassic, with average composition of $\text{An}_{43}\text{Ab}_{55}\text{Or}_2$. Rounded grains of magnetite and augite ($\text{Wo}_{42}\text{En}_{34}\text{Fs}_{24}$) are the sole remnants of ferromagnesian minerals in the Surtsey xenoliths. Xenoliths from Snaefellsnes, and rare fragments from Surtsey, are granodiorites, with up to 25% K-feldspar in the mode, as reflected in their higher K_2O (ref. 28). The Icelandic

plagiogranite xenoliths are in varying stages of fusion, ranging from trondhjemites with minor intergranular glassy films, to white frothy blocks consisting largely of very vesicular glass with minor relict quartz and plagioclase. Microprobe analyses of intergranular glasses in xenoliths ejected in the Surtsey 1963 eruption and Askja 1875 eruption (Table 1) show the general similarity between these partial melts and Icelandic rhyolites. The similarity in composition of partial melt in the 1875 Askja plagiogranite xenoliths and the rhyolitic tephra which brought these fragments to the surface (Table 1) is particularly persuasive evidence of an origin of the Askja acid magma by fusion of plagiogranites at depth.

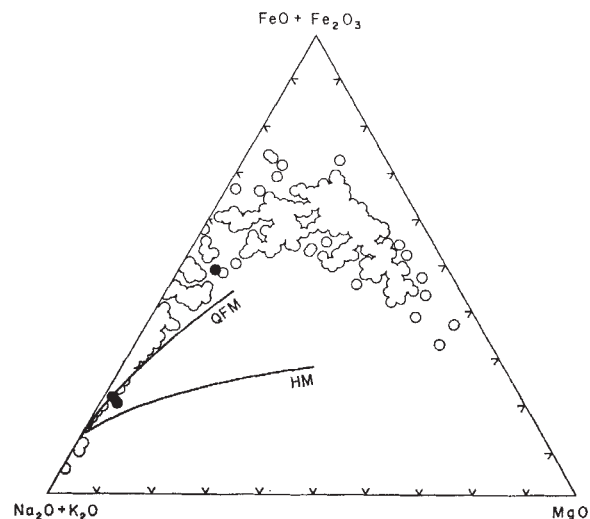


Fig. 2 Chemical variation in Icelandic volcanic rocks. Solid lines are the compositional trends of partial melts generated by anatexis of Hawaiian basalt at 5 kbar water pressure at the QFM and HM buffers¹⁶. Solid symbols mark the compositions of partial melts in plagiogranite xenoliths from Iceland (Table 1).

In Table 1 the composition of Icelandic plagiogranite xenoliths is compared with diorites from the Mid-Atlantic Ridge²⁷ and plagiogranites from ophiolite complexes¹⁹. It was suggested above that plagiogranites were formed during fractional crystallisation in magma chambers within the oceanic layer under Iceland. Direct evidence in support of this hypothesis is lacking, but it is, however, consistent with the high geothermal gradient²⁸, the ejection of cumulate gabbro nodules in Iceland^{20–31}, the geophysical structure of the lower crust, and the evidence from ophiolite complexes.

Plagiogranites in ophiolites

Most workers agree that ophiolites represent fragments of ancient lithosphere and thus provide important clues to the understanding of younger crust at modern spreading centres. Plagiogranites containing predominant zoned sodic plagioclase and quartz, with minor ferromagnesian minerals and rare K-feldspar, are a subordinate but persistent component of ophiolite sequences, occurring as discrete intrusive bodies of diffuse facies in upper part of layer 3 (refs 17, 18, 19, 22, 24, 25, 32). In the Vourinos ophiolite complex, for example, layered gabbros of the mafic zone grade upwards by increase in hornblende and quartz and decrease in pyroxenes into a 1 km thick massive layer of quartz diorite and hornblende diorite, making up over 6% of the complex¹⁹. Similar plagiogranites and other leucocratic rocks have also been dredged from the Mid-Atlantic Ridge^{27,33,34}, most notably 96 km west of the

Table 1 Composition of Icelandic plagiogranite xenoliths and their partial melts, and plagiogranites from ophiolites and the Mid-Atlantic Ridge

	1	2	3	4	5	6	7	8	9	10	11
SiO ₂	61.60	61.97	72.38	69.94	72.47	73.70	72.45	76.77	76.87	73.63	73.5
TiO ₂	0.22	0.94	0.45	0.25	0.33	0.38	0.40	0.52	0.42	0.81	0.31
Al ₂ O ₃	23.81	16.00	13.33	16.91	14.17	13.94	12.58	11.81	11.89	12.33	13.3
Fe ₂ O ₃	0.38	3.22	3.00	1.26	1.85						1.44
FeO	2.20	3.57	1.85	2.64	1.19	1.72*	4.88*	1.72*	1.62*	3.27*	1.81
MnO	0.02	0.09	0.04	0.09	0.08	0.03	0.01	0.03	0.01	0.01	0.08
MgO	0.25	2.43	0.38	0.94	1.39	1.18	0.73	0.51	0.35	0.66	0.33
CaO	6.00	3.24	2.95	2.46	1.48	2.75	3.42	0.91	0.60	2.33	1.64
Na ₂ O	5.90	5.55	3.77	3.14	5.55	4.30	4.70	3.90	3.90	4.18	4.46
K ₂ O	1.12	0.75	1.37	0.95	0.24	1.40	0.49	2.90	3.60	2.46	3.03
H ₂ O	0.46	1.28	0.30	1.20	0.90	0.70					
P ₂ O ₅	0.02	0.22	0.05	0.07	0.06	0.04	0.19	0.20		0.12	0.11
	99.98	99.26	99.87	99.85	99.71	100.14	99.85	99.27	99.25	99.80	100.01

1, Trondhjemite xenolith (1166) from Surtsey 1963 eruption²⁶. 2, Diorite dredged from Mid-Atlantic Ridge at 45°N (ref. 27). 3, Trondhjemite xenolith (1165) from Surtsey 1963 eruption²⁶. 4, Quartz diorite from the Vourinos ophiolite complex, Greece¹⁹. 5, Diorite dredged from Mid-Atlantic Ridge at 45°N (ref. 27). 6, Highly fused frothy xenolith from Surtsey 1963 eruption²⁶. 7, Partial melt of trondhjemite xenolith (1352) from Surtsey 1963 eruption. 8, Partial melt of quartz diorite xenolith (AS-26/1) from Askja 1875 eruption. 9, Partial melt of quartz diorite xenolith (AS-15) from 1875 Askja eruption. 10, Glass composition of rhyolitic tephra from the 1875 Askja eruption⁵⁰. 11, Average composition of 58 Icelandic acid rocks⁵¹. Values given are percentages.

*All iron reported as FeO.

ridge axis at 45°N. These quartz diorites and hornblende diorites are closely comparable with plagiogranites from ophiolites, both in chemistry and petrography (Table 1). The occurrence of plagiogranites in ophiolites and oceanic crust is best accounted for by a process of crystal fractionation of tholeiitic melts, operating in magma chambers within the oceanic layer^{18,19,27}. This concept is supported by the similarity in ⁸⁷Sr/⁸⁶Sr values of plagiogranites and associated gabbros^{32,33}. The negative Eu anomalies observed in plagiogranite REE patterns further show that plagioclase extraction has an important role in the differentiation process^{24,36}. The occurrence of plagioclase, olivine and clinopyroxene-cumulates or layered gabbros at the base of the mafic zone in ophiolite sequences is further evidence of crystal fractionation within layer 3.

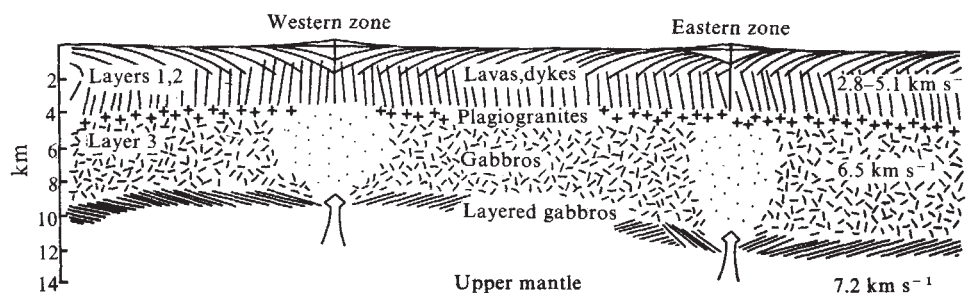
Petrological model of the Icelandic crust

The layered structure of the Icelandic crust is broadly similar to that of the ocean floors, but differs in two important details. The Icelandic crust varies from 8 to 15 km—approximately two to three times the thickness of normal oceanic crust³⁷. Much of this thickening is due to layer 2 and has been attributed to higher rate of lava production from the Icelandic mantle plume than on normal ocean ridges^{38,39}, combined with relatively slow spreading rate. Layer 3 under Iceland is also thicker than in normal oceanic crust, and differs significantly in having lower average P-wave velocity (6.5 km s⁻¹) than the oceanic layer (6.73 km s⁻¹)³⁷. This lower velocity can only partly be explained by higher temperatures and can also be attributed to higher proportion of differentiated rocks. The inverse relationship between thickness of the oceanic

layer and spreading rate²⁰ has been attributed to more efficient diffusion of interstitial melts in cumulates forming at the base of the crust, resulting in more extensive differentiation and secondary enlargement of the oceanic layer in slow-spreading regions such as Iceland³². Thus the differences in crustal structure between Iceland and normal ocean ridges may be a matter of degree rather than differences in character.

The proposed petrological model of the Icelandic crust is shown in Fig. 3. Layers 1 and 2 (2.8–5.1 km s⁻¹) are equated mainly with downwarped basalt lavas, dipping towards the long-lived western volcanic zone, grading into a lava-dyke complex with depth^{25,40}. Layer 3 is equated with gabbros, associated cumulate layered gabbros and leucocratic differentiates, along the lines suggested by Cann²⁵ for the structure of the oceanic layer. This part of the crust is envisaged as forming from crystallisation of basaltic magma in magma chambers within layer 3, principally under the rift zone flanks, where crystal settling in lateral parts of the magma chamber will give rise to layered gabbros at the base of the crust. During episodes of rifting and magma injection into the crust, such as the current episode in the north-eastern volcanic zone⁴¹, fractionation of the basaltic magma will be limited in extent. Between episodes of magma input, however, differentiation will proceed by crystal settling of calcic plagioclase and minor clinopyroxene and olivine in stagnant cooling lateral zones, distant from the axial zone of magma injection, resulting in the formation of differentiates solidifying as quartz-diorites, diorites and trondhjemites above gabbros in layer 3. Differentiates are unlikely to form in the active zone of basaltic magma injection immediately

Fig. 3 Diagrammatic model of the crust in southern Iceland, based on the seismic structure³⁷, geological evidence of the lava pile⁴⁰ and modifications of the oceanic crust model of Cann²⁵. The younger eastern rift zone has transected older crust, resulting locally in generation of rhyolite magmas from the partial melting of plagiogranites within layer 3 by ascending basaltic magmas.



under a rift, and are envisaged as forming rather within the upper part of layer 3, some kilometres away from the axial zone.

Generation of rhyolite magma

In Iceland the distribution of rhyolites is closely associated with central volcanoes, where they make up to 20% of the exposed volcanics in many cases⁸. As pointed out by Sigvaldason⁷, the great majority of rhyolite-producing central volcanoes of Late Quaternary to Recent age occur in volcanic zones which have dissected and are underlain by older crust, such as the eastern rift zone and the Snaefellsnes volcanic zone. Rhyolites are, on the other hand, altogether absent from the rift zone of Reykjanes peninsula⁴², a region underlain by young crust. The distribution of plagiogranite xenoliths follows a similar pattern. They are most numerous in the Snaefellsnes zone, where recent volcanism has penetrated 5–13-Myr-old crust⁴³, and in the eastern zone, from Surtsey to Askja. Saemundsson⁴⁴ has inferred that the northern part of the eastern zone was established in its present position 4 Myr ago, following rift-jumping from the west, on to 8-Myr-old crust in the east.

It is proposed that the majority of rhyolites erupted in the Quaternary to Recent volcanic zones in Iceland are generated by melting of plagiogranite within layer 3 during episodes of injection of basaltic magma into a rift zone recently established within older crust. The availability of plagiogranites for this melting process is, however, limited to regions of older crust. Rhyolitic volcanism of this type is then restricted to regions of rift jumping, where a younger rifting episode transects older crust containing a differentiated gabbroic sequence with overlying plagiogranites. The structural and time relationships of individual rhyolitic centres are rarely known in sufficient detail to test this model. The well-studied Hallarmuli central volcano in western Iceland is a good case in point, however. Johannesson⁸ has shown that the earliest volcanics of this centre were acid ignimbrites erupted 6 Myr ago, on a 12.5-Myr basaltic crust. The model is also consistent with available geochemical evidence, such as measurable differences in ⁸⁷Sr/⁸⁶Sr ratio between rhyolites and field-associated basalts in the Torfajökull centre⁵. O'Nions and Gronvold⁵ pointed out that the present-day ⁸⁷Sr/⁸⁶Sr ratio of the rhyolites could be accounted for by postulating a 16-Myr-old source with initial Rb/Sr ratio of approximately 0.8. The observed range in Rb/Sr ratios of Icelandic plagiogranite xenoliths varies from 0.04 to 2.1 (ref. 26) and demonstrates the availability of such a source within older regions of the Icelandic crust. The large negative Eu anomaly in rare earth element (REE) patterns of Icelandic rhyolites⁵ is also comparable with the Eu anomaly in plagiogranites from ophiolite complexes^{24,32,36}, but the meagre data indicate lower overall REE abundances in the latter.

Implications of the proposed two-stage model for the origin of Icelandic rhyolites and petrological structure of the Icelandic crust are diverse. It is emphasised that the high volume of rhyolites erupted at the surface is not anomalous when considered in the framework of fractional crystallisation within layer 3. Furthermore, it is likely that the volume of rhyolites exposed at the surface in Iceland is not representative of their total volume in the crust as a whole, due to the possibility that the less dense rhyolitic melts will be preferentially concentrated at higher levels in the crust⁴⁵. If it is assumed, for purpose of illustration, that layers 1 and 2 are 4 km and layer 3 is 8 km thick, and that 10% of layers 1 and 2 are rhyolite, then formation of a salic differentiate residuum amounting to 5% of layer 3 would be required to form a plagiogranite source for the rhyolites. An important corollary of this model is that the basalt magmas which appear at the surface will in many cases have been modified by extensive fractional crystallisation², as the generation of leucocratic rocks within layer 3 is envisaged as a result of

differentiation of primary basaltic magma rising into the Icelandic crust. Also, it is likely that the heat required for partial melting of plagiogranite within older crustal regions is provided by extensive crystallisation of basaltic magmas in proximity with the leucocratic rocks. Thus basaltic lavas, associated with rhyolites which have originated in this way, are likely to be fractionation products of more primitive melts.

The mechanism envisaged for generation of Icelandic rhyolites is likely to be equally applicable to other oceanic hot-spots where rift-jumping has occurred. An appropriate example is the Easter Island region, where eastward jump of the Nazca-Pacific plate boundary has been documented⁴⁶. Taken together with the structural evidence, the marked chemical discontinuity between Easter Island rhyolites and associated basalts and the differences in ⁸⁷Sr/⁸⁶Sr ratio⁴⁷ suggest that Easter Island rhyolites may also owe their origin to fusion of plagiogranites within layer 3, as a consequence of rift-jumping. Similarly in the Azores region a marked bimodal compositional distribution occurs between basalts and contemporaneous salic peralkaline lavas⁴⁸ where comendites have significantly higher ⁸⁷Sr/⁸⁶Sr ratios than associated basalts⁴⁹. Volcanism in the Azores islands is related to easterly trending fracture zones transecting older crust, generated at the Mid-Atlantic Ridge to the west. Thus the structural framework and geochemical evidence are also consistent with generation of the salic rocks in the Azores by remelting of plagiogranites at depth. Salic peralkaline lavas are volumetrically insignificant but widespread amongst oceanic islands. If their genesis can be interpreted in terms of partial fusion of plagiogranite within the oceanic layer, then is the cordierite–mullite refractory assemblage in the Icelandic xenoliths providing a clue to the origin of the peralkaline condition?

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Structure of nucleosome core particles of chromatin

J. T. Finch, L. C. Lutter, D. Rhodes, R. S. Brown, B. Rushton, M. Levitt & A. Klug

MRC Laboratory of Molecular Biology, Hills Road, Cambridge, UK

Crystals have been obtained of nucleosome cores and analysed by X-ray diffraction and electron microscopy. The core is a flat particle of dimensions about $110 \times 110 \times 57 \text{ \AA}$, somewhat wedge shaped, and strongly divided into two 'layers', consistent with the DNA being wound into about $1\frac{3}{4}$ turns of a flat super-helix of a pitch about $28 \text{ \AA}$. The organisation of the DNA can be correlated with the results of enzyme digestion studies. A change in the screw of the DNA double helix on nucleosome formation can be deduced.

THERE is a good deal of evidence that, as proposed by Kornberg¹, chromatin consists of repeating units, termed nucleosomes, containing a fairly well defined length of DNA² (200 base pairs in rat liver³) associated with an octamer aggregate of the histones containing pairs of each of the four main types (for review see ref. 4). The fifth histone, H1, is also associated with the nucleosome, but in a manner which is not clear. Enzyme digestion studies on nuclei, using micrococcal nuclease, have shown that while the DNA content of nucleosomes is often about 200 base pairs, quite large variations are found according to the species or tissue investigated. But it has been found that further nuclease digestion produces a 'core' particle⁵⁻⁹ containing the same number, ~140 of base pairs in all cell types so far investigated. The rest of the DNA in the repeat length not included in the core is thought of as a linker between core particles. We report here the crystallisation of nucleosome core particles, and X-ray and electron microscopic studies on the crystals which have led to a low-resolution map of the structure. By combining this result with data from enzyme digestion studies on the DNA in nucleosomes and general considerations on the bending of DNA, we can obtain a picture of the organisation of DNA in a nucleosome core and a rationale for this organisation.

The work began following the discovery in this laboratory by Noll and Kornberg in 1974-75 that, on more prolonged digestion with micrococcal nuclease, the repeating unit of rat liver chromatin is broken down progressively from 200 base pairs of DNA. They found a pause in the digestion at the 160-base-pair stage and a longer one at 140 base pairs, between which stages histone H1 is released⁹. Early experiments on crystallising particles at the 160-140-base-pair stage were not successful, probably due to the presence of residual H1. It was not until the development of a method for the preparation of homogeneous nucleosome cores on a large scale for the purpose of biochemical studies (L.C.L., in preparation) that crystals could be regularly and reproducibly grown. Crystallisation of nucleosome cores has been reported previously⁷ but no analysis has been made.

Intact nucleosome cores have been crystallised and studied but we found that the best crystals so far obtained, which diffract to spacings of about $20 \text{ \AA}$, come from particles which have had some of their histones cleaved by proteolysis. For the reasons given later we think that the core particles have not in consequence suffered

any significant change in structure, and that the results obtained are generally valid. The unit cell is large and contains three particles in the asymmetric unit, which then has a molecular weight of about 600,000, so that the solution of the structure by X-ray methods is a formidable task. For this reason the X-ray analysis has been carried out in conjunction with electron microscope studies on the crystals, and related aggregates, using methods of image reconstruction developed in this laboratory¹⁰. We have solved the three centro-symmetric projections of the crystal structure, and because one of them is down an axis looking through the width of only one particle, a fairly clear picture of the overall structure of the nucleosome core has emerged.

Preparation and characterisation of nucleosome cores

A detailed description of the preparation will be given elsewhere (L.C.L., in preparation). Essentially it involves preparation of nuclei from rat liver² and production of long chromatin from these nuclei by digestion with micrococcal nuclease¹¹. The long chromatin is then passed over a Sepharose 4B column equilibrated with 0.45 M NaCl to remove histone H1 as well as non-histone proteins, redigested with micrococcal nuclease and chromatographed on Sepharose 4B to produce a preparation of monomer core particles. The DNA of these cores is nominally 140 base pairs in length⁹. Polyacrylamide gel electrophoresis of the DNA from this preparation shows a single band, as seen in Fig. 1. When compared with the DNA from a DNase I digestion of chromatin, which contains a series of bands which differ by 10 bases¹², it can be seen that the monomer DNA has length distribution similar to that of a single DNase I-produced band; that is, less than ± 3 bases. The monomer preparation contains no histone H1 and usually contains normal amounts of histones H2A, H2B, H3 and H4 (Fig. 2).

These intact nucleosome cores almost invariably gave microcrystals as described below. But, the largest single crystals (Fig. 3) were obtained on occasions when the histones in the core particles showed extensive proteolysis (Fig. 2c, d). In most of these preparations the H3 and H2B bands have almost completely disappeared and new low molecular products are present. When this proteolysis occurred an endpoint seemed to be reached, as judged by the gel patterns. We have not investigated the nature of the products, but we believe that these partially proteolysed nucleosome cores are not much changed in their gross structure from the intact ones. First, the sedimentation constant of the proteolysed particle in 100 mM NaCl is hardly changed from the intact material (10.3S against 10.5S: we thank Dr P. J. G. Butler for these measurements). Second, brief DNase I digestion shows a pattern of bands every 10 bases (Fig. 1) which is very similar in its intensity distribution to that from intact particles, except that the rate of digestion of the proteolysed particles is higher. Moreover the relative frequencies of cutting at the individual sites, as determined by end-labelling experiments (see below), remain very similar for the proteolysed particles. Third, the crystal structures of the

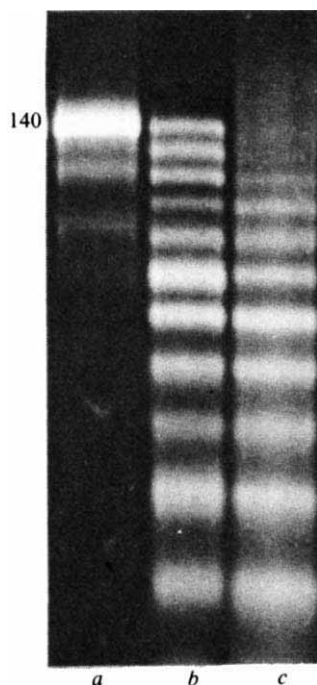


Fig. 1 Polyacrylamide gel analysis of DNA in single stranded form from *a*, 140-base-pair nucleosome cores which gave the large crystals used for X-ray work (and in which some of the histones had been proteolytically cleaved, Fig. 2*c*); *b*, preparation (*a*) digested with DNase I; *c*, rat liver nuclei digested with DNase I. The DNase I digestion and gel electrophoresis were carried out as described in refs 3 and 9.

two are closely related. Furthermore, various preparations showing somewhat different patterns of proteolysis (for example, Fig. 2*c*, *d*) crystallised in identical forms, but with occasional differences in habit. For these reasons it is possible that the smaller fragments of the proteolysed histones (~1,000 to 3,000 molecular weight) which can be seen on the gel patterns are still present in the particles which have crystallised. If so, then the histone molecules on the core particle may not be much changed in tertiary structure, although some covalent linkages have been cleaved. This question is under investigation.

Crystallisation methods

The general methods used were similar to those described for crystallisation of various transfer RNAs in this laboratory^{13,14}. The 'monomer' peak from the Sepharose column was concentrated to about 3–6 mg ml⁻¹, and this solution was dialysed against a buffer containing 10 mM Tris-HCl, pH 7.4, 0.2 mM EDTA, 0.1 mM phenylmethylsulphonyl fluoride and 0.3 mM azide. Crystals of the nucleosome cores have been obtained at both 4 °C and room temperature using a variety of components in the crystallisation mixture, but not enough experiments have been done so far to provide a phase diagram as complete as that for tRNA (ref. 14). The final concentration of the nucleosomes in the crystallisation mixture ranged from 0.5 to 2 mg ml⁻¹. Microcrystals and small single crystals were grown in the presence of 0.4–0.6 mM spermine tetrahydrochloride, or 3–4 mM MgCl₂ plus 0.2 mM spermine or else at 10 mM MgCl₂, 1.5 mM spermine and NaCl up to concentrations of 100 mM in 10% (w/v) hexan-1,6-diol. Small crystals were also grown in the presence of polyethyleneglycol 6000 (5–10%, w/v) which had the effect of reducing somewhat the concentration of Mg²⁺ and spermine required.

The crystals were grown either in drops in glass cavity slides, equilibrated against the organic solvents or in small

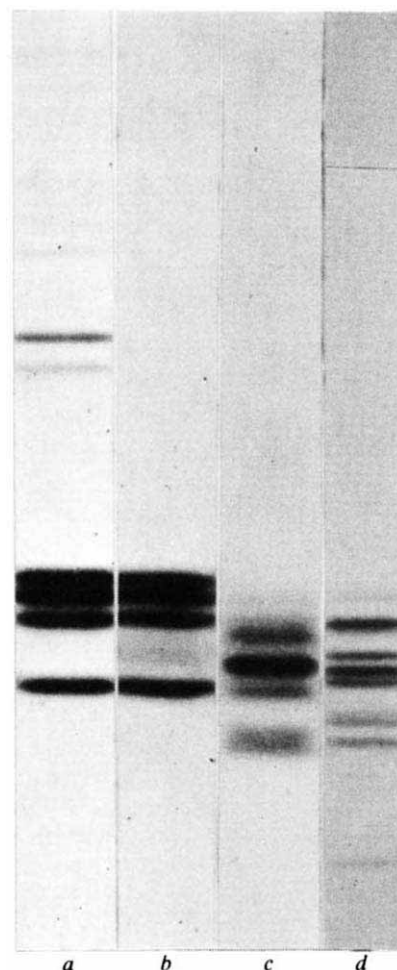


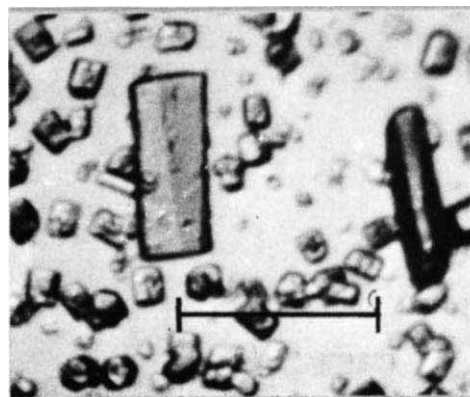
Fig. 2 Histone composition of *a*, rat liver nuclei; *b*, crystals of nucleosome cores with intact histones; *c* and *d*, crystals of two preparations of proteolytically cleaved nucleosome cores. The sodium dodecyl sulphate-polyacrylamide gel analyses were carried out as in ref. 42.

stoppered glass tubes containing the appropriate mixtures. In both the vapour diffusion and direct addition methods, crystallisation occurred in 1–4 d.

Crystal packing

No large single crystals of intact material have yet been grown, but in the conditions described above microcrystals were obtained. X-ray diffraction 'powder' diagrams from these show three clear rings of spacings at 96, 55 and 37 Å and a fainter ring at about 29 Å. The first three spacings

Fig. 3 Crystals of nucleosome cores. The two larger ones are typical of those used for the single crystal X-ray diffraction work. Scale bar, 0.2 mm.



index on a two-dimensional hexagonal cell of side $110\text{ \AA}$, and correlate well with electron microscopic observations on the crystals which show extensive areas of a hexagonal arrangement of particles with this same cell side (Fig. 4). Such close packing is natural for particles with a circular shape. Although the micrograph showed these crystals to be several layers deep with the particles in register, so that the nucleosomes formed columns hexagonally packed, no obvious extra lines were found in the powder diagrams to indicate the inter-layer spacing. In view of the results described below, however, it seems likely that the inter-layer spacing is $57\text{ \AA}$ —and so unresolved from the $55\text{ \AA}$ ring for the basal hexagonal cell—and that the observed ring at $29\text{ \AA}$ is its second order, since it definitely does not index on the hexagonal cell.

X-ray powder diagrams from small crystals of cleaved material show rings with the same spacings as the intact material but in addition, rings at spacings, 168 , 72 and $50\text{ \AA}$, and other weaker unresolved rings. The significance of these became clear when crystals grew sufficiently large to be studied singly. To anticipate the results, these spacings reflect the fact that the cleaved core particles do not stack over each other in register but are displaced sideways to produce 'wavy' instead of straight, hexagonally packed columns. The largest crystals grew to about $200 \times 50 \times 20\text{ }\mu\text{m}$ in size and X-ray precession photographs were obtained. The X-ray patterns extend to spacings of about $20\text{ \AA}$ and the patterns in the directions of the cell axes are shown in Fig. 5. At this stage we cannot tell whether the limit to the resolution is set by disorder in the crystals or by the combination of small crystal size and large unit cell. The crystals are orthorhombic of unit cell sides $a=110\text{ \AA}$, $b=192\text{ \AA}$ and $c=340\text{ \AA}$. The absence of odd index reflections along the directions of the reciprocal cell sides suggests that, at least to this resolution, the space group is $P2_12_12_1$. Most of the X-ray data out to spacings of $25\text{ \AA}$ have been collected and a three-dimensional Patterson function calculated, the features of which are consistent with this space group.

The ratio of the cell sides $b:a$ is 1.74 , very close to $\sqrt{3}=1.73$, suggesting a pseudo-hexagonal packing in the (001) plane, probably of columns spaced $110\text{ \AA}$ apart. The high intensity of the 006 and 0012 reflections indicate strong subdivisions along the c axis of 57 and $28.5\text{ \AA}$, but there is no indication of a $110\text{-\AA}$ subdivision in this direction in either the X-ray pattern or the Patterson. We therefore conclude that the units packed along a column are spaced $57\text{ \AA}$ apart, and have a strong bipartite character in this direction. On this basis there would be 12 of these units per unit cell and these can be identified as nucleosome cores, since density measurements, although difficult with such small crystals, set

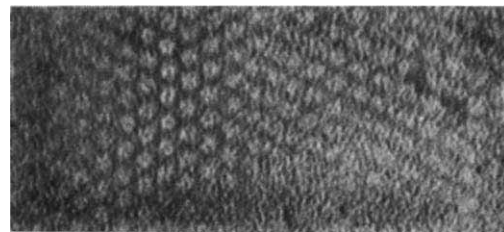


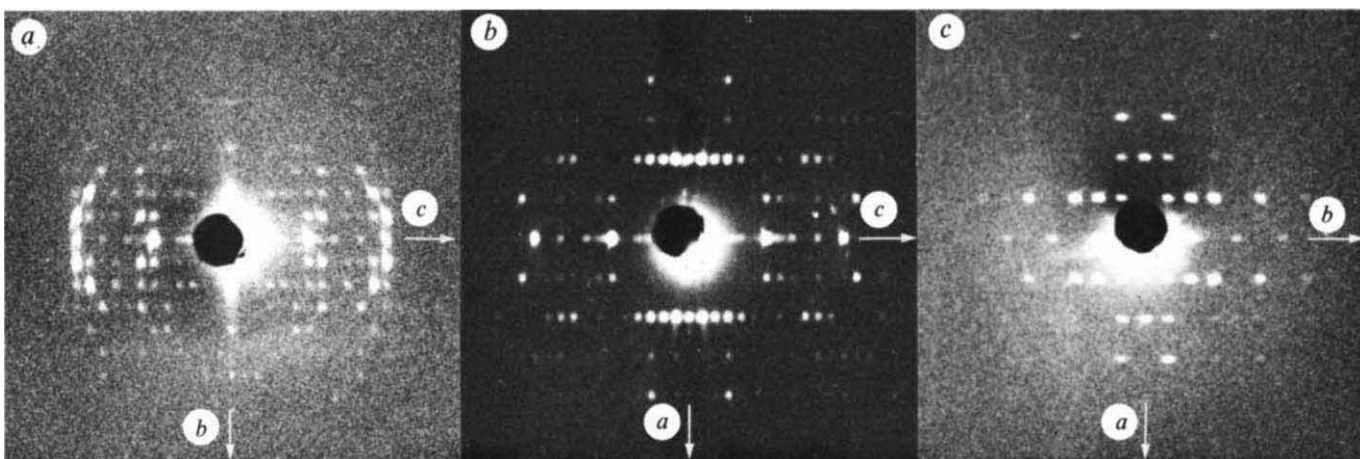
Fig. 4 Electron micrograph of crystal of nucleosome cores with intact histones, negatively stained with uranyl acetate. The cores are hexagonally packed with an interparticle distance of $110\text{ \AA}$. The dark centres indicate a lower density there than at higher radii. ($\times 300,000$).

limits of 9 and 14 for the number of particles in the cell. Twelve particles per unit cell means 3 in the asymmetric unit, which then has a molecular weight of $600,000$.

Both the $[100]$ and $[010]$ X-ray photographs show strong reflections in the neighbourhood of the 006 and 0012 reflections, which, together with other features of the patterns, suggest that the columns of nucleosomes are not straight. The simplest interpretation¹⁵, confirmed later by electron microscopy, is that in the $[010]$ projection, the columns of nucleosomes appear as approximate sine waves of period $c=340\text{ \AA}$ and amplitude about $14\text{ \AA}$, adjacent waves being approximately in phase. In the $[100]$ projection, the columns are also sinusoidal with a period of $340\text{ \AA}$ and amplitude about $8\text{ \AA}$, but adjacent waves are out of phase. Together the two projections imply that in three dimensions the line of particles in the z direction follows a sine wave lying in a plane rather than a helix. The projections of the sinusoidal columns down the z axis are elongated and there are two different directions of elongation in the unit cell. Hence although the centres of the projected columns lie on a hexagonal lattice, the packing is only pseudo-hexagonal, accounting for the presence in the $[001]$ X-ray photograph of reflections with $h+k$ odd.

The chief differences in the 'powder' patterns from the intact and cleaved material can now be explained. In the crystals of the intact cores, the particles stack in register in the c direction, leading to straight rather than wavy columns (hence the absence of the 011 reflection at $168\text{ \AA}$). These columns are cylindrical when projected along the c axis, hence at low resolution the packing appears indistinguishable from hexagonal, whereas the wavy columns of the cleaved particles give an elliptical projection and hence stronger departures from hexagonality, exemplified by the 120 reflections at $72\text{ \AA}$. The straight columns of the intact material are divided into

Fig. 5 X-ray precession photographs of crystals of nucleosome cores taken in the directions of the unit cell sides a , $[100]$; b , $[010]$; c , $[100]$.



57 Å and 28 Å spacings in the same way as is the cleaved, corresponding to the nucleosome spacing and its halving. This has been confirmed by recent X-ray photographs of single crystals of intact core particles which show the strong 00 l reflections corresponding to the thickness and bipartite nature of the nucleosome core. The c -axis repeat is now only 110 Å, corresponding to two nucleosomes 55 Å 'high', while the a and b axes remain the same.

Electron density map

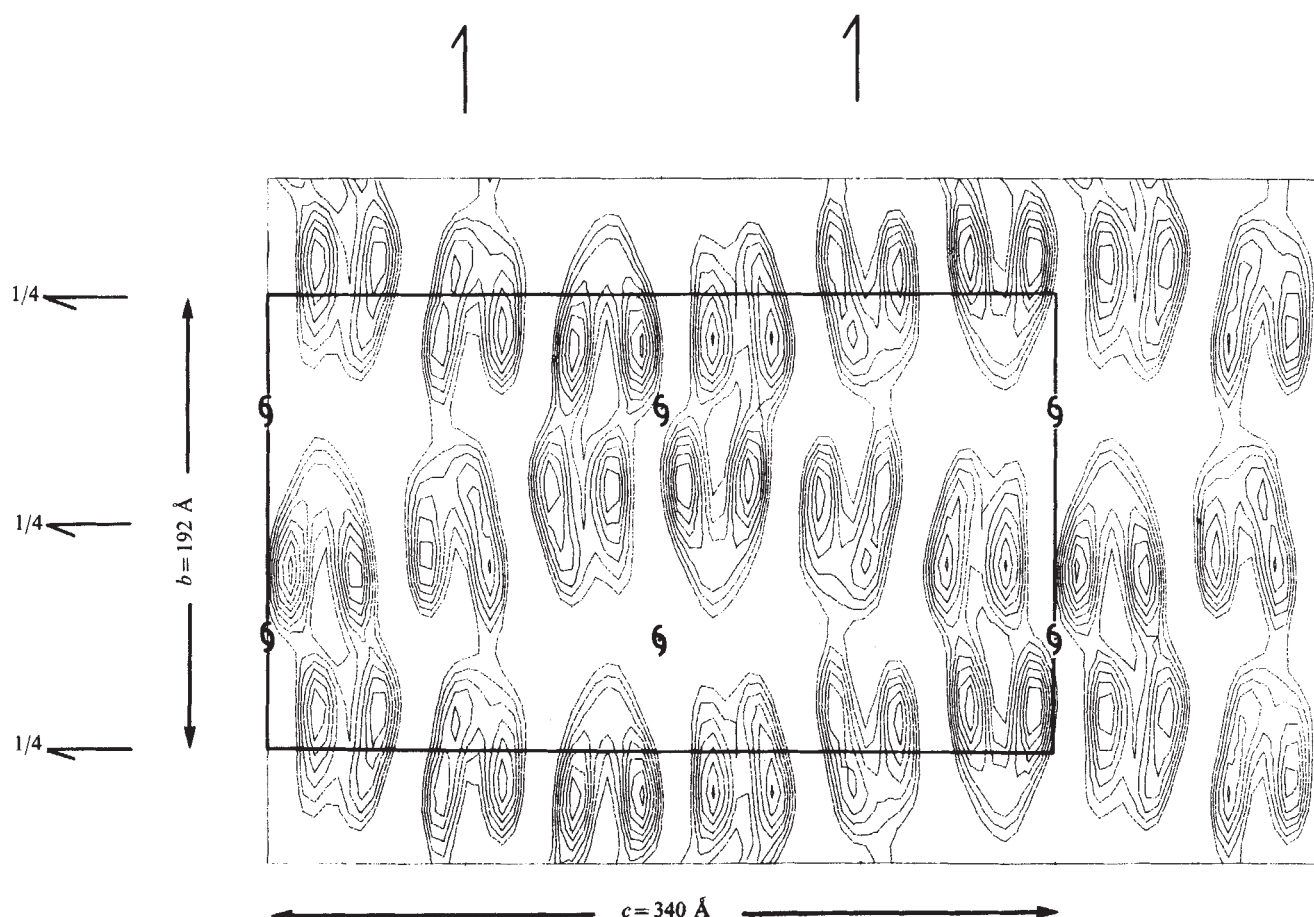
The packing deduced from the X-ray data has been confirmed by electron microscopy. Patterns which can be identified with views of the crystal in the direction of the cell sides have been found in electron micrographs of fragments of crystals. Although there is considerable shrinkage particularly in the 340-Å direction (vacuum dried crystals visibly shrink some 10%–20% in this direction) the distribution of intensity in optical diffraction patterns made from the micrographs agree well with the low-angle part of the corresponding X-ray patterns. We have found images corresponding to the three principal projections, selected and processed the best of them using optical and computer methods for image analysis¹⁰. We have used the phases (signs in this case) of the reflections determined by computing the Fourier transforms of the images, with the amplitudes derived from the X-ray patterns to calculate electron density maps of the principal projections of the crystal structure. This analysis did not give the signs of some of the outermost strong reflections and we therefore assigned signs to make the three crystallographically independent particles in the unit cell as alike as possible. This use of 'non-crystallo-

graphic symmetry' is a compensating advantage of working with a large unit cell. A fuller account will be published elsewhere¹⁵.

In the [100] and [001] projections there is considerable overlap of units at different depths in the unit cell although in the former the sinusoidal columnar nature of the packing is evident and in the latter the pseudo hexagonal packing of the columns. The least overlap occurs in the [010] projection, the map of which is shown in Fig. 6. Wavy columns of particles, that is nucleosome cores, about 57 Å apart, are clearly visible. The density in each particle is strongly divided into two but the two halves are not always parallel to each other, so that the particle itself looks wedge shaped. Indeed the run of particles in an arc of the sine wave is reminiscent of the arrangement of voussoirs in an arch, but the map gives the impression that the three crystallographically independent cores do not all have quite the same rotational setting about the c direction.

The electron micrographs we have obtained of the crystals do not directly show much detail beyond the arrangement of the core particles. We have therefore searched for specimens which would show more details of the internal structure and so be compared with the map shown in Fig. 6. To this end we have set up specimens for crystallisation, examined them in the electron microscope before visible crystals appeared and found wavy columns of particles. Figure 7 shows some nested columns, similar to the packing which would exist in a section of the crystal perpendicular to the y axis and one layer thick. Along the columns, associated pairs of 27-Å striations are often clearly evident, but the nature of the

Fig. 6 Electron density map of the projection of the crystal of nucleosome cores in the direction of the a axis of the unit cell. The nine strongest amplitudes in the [100] X-ray diagram (Fig. 5a) out to spacings of 25 Å were included. The signs of seven of these were taken from the calculated Fourier transform of an electron micrograph of a crystal in the corresponding orientation. The signs of the remaining two amplitudes were chosen to make the three crystallographically independent particles in the asymmetric unit most nearly equivalent.



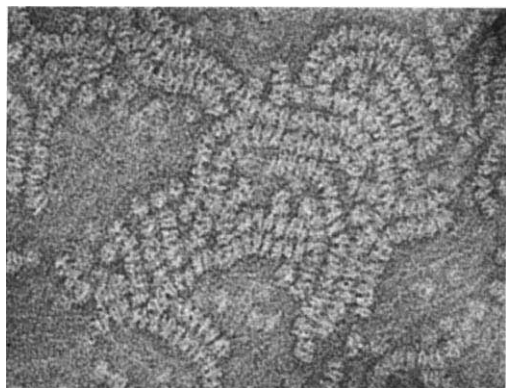


Fig. 7 Electron micrograph of columns of nucleosome cores formed after a short time in the conditions of crystallisation, negatively stained with uranyl acetate ($\times 300,000$). The wavy columns correspond to those along the *c* axis in the crystal (Fig. 6). They are about 100 Å in diameter and consist of stacks of bipartite nucleosome cores spaced about 55 Å apart. The two halves of the particles do not always appear parallel and are often V-shaped especially at bends in the columns. The fine details along the two halves of a particle are often related by mirror symmetry consistent with views of a particle with several radially directed pseudo-dyad axes.

pairing varies along the column. Sometimes the associated pair of striations are parallel but often they are not, giving a wedge-shaped appearance to the bipartite unit. In all of these cases, however, there is a strong tendency for the two halves to be related by approximate mirror symmetry, consistent with views at right angles to a twofold axis, or pseudo twofold axes, such as might arise from the arrangement of eight similar histone molecules about a true dyad.

Relation to small-angle scattering studies in solution

The flat shape seen for the nucleosome core is consistent with the determination of the volume and surface area of (intact) core particles by a model-independent analysis of small angle X-ray scattering data in solution (A. Tardieu and L. Sperling, in preparation). Whereas the radius of gyration (determined from the very low-angle data) would correspond to a uniform sphere of radius 57.6 Å, the surface area (determined from analysing the scattering curve to a resolution of 20 Å) would correspond to a sphere of radius 72 ± 5 Å, suggesting a shape departing markedly from the spherical.

There have been earlier proposals for a disk shape for the nucleosome core^{16,17}. The model calculations of the Searle group¹⁸ to explain their low angle X-ray and neutron scattering data in solution (to a resolution of about 30 Å) show that a flat particle fits the data well; and they have proposed a model for the core of the type we have found, although scattering in solution which yields spherical averages of intensities, can never prove a model. Only three dimensional data can resolve superposed intensities and give direction to dominant spacings observed.

Thus it can be seen that the 38-Å and 27-Å bands observed in X-ray scattering from nucleosome cores in solution (A. Tardieu and L. Sperling, in preparation), and also of course in whole chromatin, come from directions at right angles to each other. It is very likely that the 38-Å reflection which occurs in the plane of disk has a contribution from the second maximum of a Bessel function corresponding to the radius of the DNA, the first maximum being at 70 Å, which in the X-ray data appears as a broad shoulder superposed on the steeply falling part of the 'shape' scattering curve. But the 70-Å

maximum shows up clearly in the spherically averaged 'internal' scattering function deduced from neutron scattering using contrast variation¹⁹. The 27-Å band comes predominantly from internal structure along the axis of the short cylinder, corresponding to the spacing of the turns of the DNA superhelix. Again, this fluctuation of density is evident in the 'internal' structure function¹⁹. There is therefore no gross difference in structure between nucleosomes in solution and in the crystal.

There are also other obvious implications for interpreting the X-ray patterns from whole chromatin. Thus the crystal data shows that the well known '55-Å reflection' of chromatin in fact occurs in two directions at right angles (in the neighbourhood of the reflections 200 and 006) and has a quite different origin in the two cases, the first as a second order of the side to side spacing of the cores, and the second as the distance of contact between their flat faces. The first order of the side to side spacing at 110 Å is cancelled by the packing in the crystal but presumably corresponds to the '110-Å reflection' of chromatin.

Structure of nucleosome core

The most informative of the three projections we have obtained is that down the *a* axis (Fig. 6) because one is looking only through a single layer of particles with a little overlap at the edges. The nucleosome core is clearly a flat particle of dimensions $57 \times 110 \times 110$ Å but seems to have a wedge shape consistent with its being formed of something less than two superhelical turns of DNA wound on a flat histone core. Because of its shape we call it a platysome. Again, as described above for the electron micrographs, the two halves of a platysome in the map are related by approximate mirror symmetry, consistent with a dyad or pseudo-dyads in the plane of projection. This is consistent with a model in which two turns of DNA (strictly parts of two turns) run along the outside of a histone core which may itself consist of two layers or turns. At the present resolution, and also because one is dealing with a projection, one cannot distinguish DNA and protein, but the map is not consistent with a structure in which the DNA lies in a deep groove formed by the histones. Rather, the DNA is fitted on to the periphery of the histone octamer rather like a helical tyre. The location of the DNA on the outside of the nucleosome was originally suggested by Kornberg⁷, and it has been confirmed by low-angle neutron scattering in solution that the bulk of the DNA does lie at an outer radius^{19,20}.

It is not possible at the present stage to follow the path of the DNA in the nucleosome core but the simplest assumption is that it follows a more or less regular helix. Whatever the details, the DNA is wound in a flat superhelix of pitch about 28 Å and average diameter about 90 Å (Fig. 8a). The value for the pitch comes from the side view of a particle in the electron density map (Fig. 6). The pitch of the superhelix is small enough to allow interactions between the two turns, mediated by cations and/or histone salt bridges. The diameter is fixed by the fact that the very outside diameter of a nucleosome core can at most be only 5 Å or so greater than the shortest centre-to-centre distance, which is not less than about 100 Å. The reason is that platysomes in neighbouring columns parallel to the *c* axis are staggered by only about 10 Å (Fig. 6) so there is not much interlocking which would have led to a larger diameter for the DNA. Assuming a diameter for the DNA of 22 Å the diameter for the DNA coil is about 90 Å, and if the DNA is assumed to be in the B form, we estimate there are 75–82 bases per superhelical turn.

Little can be concluded about the arrangement of histones. The dimensions of the histone core, deduced by

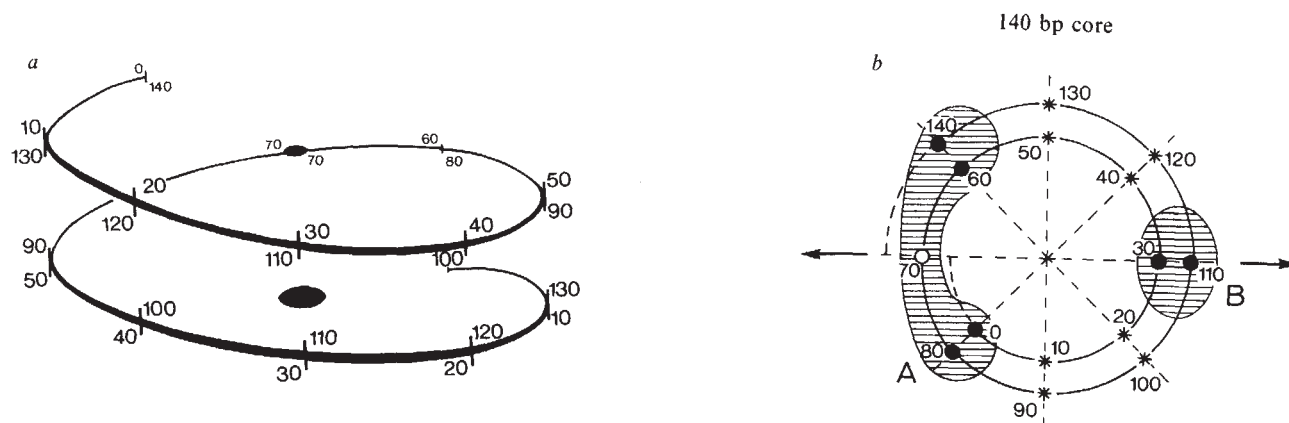


Fig. 8 *a* Diagram, drawn roughly to scale, of the $1\frac{1}{2}$ turns of the DNA superhelix proposed for the 140-base-pair nucleosome core. The top set of numbers gives the distances in bases of the DNase I cutting sites from the 5' end of one strand, and the bottom set refers to the other strand, related to the first by the dyad shown. For definiteness, the number of bases per turn is shown as 80, but the exact number is not established. *b*, The $1\frac{1}{2}$ turns of one strand of the DNA superhelix represented as a spiral to show how the supercoiling brings sites 80 bases apart close together and groups the sites of low or medium cutting frequency by DNase I into the two diametrically opposite areas A and B, shown shaded. The arrow indicates the dyad. The numbers give the distance in bases from the 5' end. ★, High frequency DNase I cutting. ●, Low (or micrococcal pauses). ○, Medium.

'subtracting' the DNA, show the octamer to be a compact particle, of packing volume similar to that found for other globular protein aggregates such as haemoglobin. It is, however, not possible to tell whether the histone molecules form a shallow helical ramp of two turns or whether there are simply two layers which are distorted or have projections on them to provide such a helical ramp. There is a striking similarity between some of the dimensions which occur in the columns of nucleosomes found in the crystals and the spacings observed in optical diffraction patterns of electron micrographs of certain histone fibres assembled without DNA²¹. In the columns in the crystal the histone cores of adjacent platysomes must be in contact and the packing may be similar to that in the histone fibres. If this is so, the 28-Å group of reflections in the crystal (compare Fig. 5a) would have a contribution from the histone cores, which would thus also tend to be divided into two.

Relation to enzyme digestion studies

We now turn to the question of the organisation of the DNA in the nucleosome core, and show how our structural results can be correlated with the results of enzyme digestion studies. The action of DNase I on chromatin or nucleosome cores is to produce a set of DNA fragments differing in size by 10 bases¹², and this was interpreted as reflecting the fact that the enzyme either cuts at points on smoothly bent DNA which are 'maximally exposed' (that is, lie furthest from the centre of the particle) or at 'kinks' in the DNA where the structure is distorted. Whatever the explanation, the important fact is that periodicity along the DNA helix is 10 bases or very close to 10. It is clear, however, that the cuts are not equally frequent every 10 bases and the assumption is that frequency of cutting reflects differential protection of different tracts of the DNA by the histone interactions. Several studies have been made of the frequency distribution by radioactively labelling an end of the DNA in the 140 base-pair core, then digesting with DNase I, and then analysing the distribution of end-label amongst the single-stranded fragments produced (refs 22 and 23, and M. Noll in preparation). Numbering in bases from the 5' end, it is found that the sites at 10, 20, 40, 50, 90, 100, 120 and 130 are readily attacked, so that cuts tend to occur at 30–40 or 80–90 bases apart. There are quantitative differences in the results of the different investigators but the local rank order among the cuts is the same.

Now as stated earlier the diameter of the DNA coil is best fitted by about 80 bases per turn, so that points on the DNA double helix 80 bases apart are closest together on the superhelix, only about 30 Å apart, and can therefore be protected, or otherwise, by neighbouring patches of histones (Fig. 8b). The positions of the most frequent cuts are consistent with this, but would not in themselves rule out a periodicity of 90 bases. The decision in favour of 80 is, however, unambiguously made by considering the least frequently cut sites, which are at positions 30 and 110 and are flanked on both sides by sites of high frequency cutting. This reasoning can be extended to include the results of micrococcal digestion studies, which show a 'pause' at the 160 base-pair stage before the more stable 140 base pair particle is produced. If the 160-base-pair particle corresponded to a structure with two complete turns, (which can be recreated by extending the DNA of Fig. 8 from the points 0 and 140 to –10 and 150), then the sites at 0 and 140 reflect places of relatively high protection of the DNA. These two positions can be correlated with the weak DNase I cuts at 80 and 60 respectively, which are 80 bases away. Such a 160 base pair particle would have its two ends 'in line' with each other and with the midpoint of the DNA. Through the midpoint would pass the putative dyad of the structure. The other end of the dyad would pass between the turns of the DNA, that is, between sites 30 and 110 (Fig. 8a).

The DNase I cutting pattern is consistent with the assumption that the nucleosome core possesses a dyad and the matter is discussed in detail elsewhere²². If there is a dyad as shown in Fig. 8a, then if a cut occurs on one strand, it is equally likely that a cut occurs at the equivalent position on the other strand, that is at the same distance from the 5' end. But since what is measured is the average of the frequency of cutting on the two strands, it is not possible to tell whether the cutting is equally apportioned between the two. Crick has pointed out that the strongest evidence for a dyad comes from the fact that there are weak (or zero) cuts, as here cuts at equivalent positions on both strands must be both zero or small and hence equal. Apart from the histone composition there are other biochemical results suggesting the presence of a dyad. It has been shown²⁴ that DNase II can cut down the DNA of a 200-base-pair nucleosome into two equal halves without disrupting the particle physically and there is electron microscopic evidence that in

appropriate conditions a nucleosome can open up into two separate half-nucleosomes²⁵.

Supercoiling of the DNA

If DNA is closed into a circle after interaction with histones in the presence of a 'DNA relaxing' enzyme, the DNA is seen to supercoil when the histones are removed²⁶. Several workers have estimated that the 'number of supercoils' is between -1 and $-1\frac{1}{2}$ per nucleosome²⁶⁻²⁸ (the minus sign indicates left-handed supercoiling). It was pointed out by Crick²⁹ that what these experiments measure is the change in linkage number (here the number of times one strand of the DNA double helix is wound around the other strand) caused by forming nucleosomes. It is not possible by the techniques used to distinguish between the separate contributions made to the change in linkage number by the coiling of the DNA into a superhelix and by any concomitant change in the local twist of the DNA double helix. Only the sum is conserved. The change in linkage number is the same as the number of superhelical turns only if the screw of the DNA double helix does not change in the local coordinate frame defined by the superhelix path.

Our results on the crystals show that as the maximum DNA radius in the core particle is about 45 Å, the 140 base pairs of DNA must make more than $-1\frac{1}{2}$ turns of superhelix. In fact we propose $1\frac{1}{4}$ superhelical turns corresponding to 80 base pairs per turn. The only way to reconcile these results with the linkage number change of $-1\frac{1}{4}$ is to conclude that the helical screw of the DNA double helix does indeed change on binding DNA free in solution on to the nucleosome. It would be possible to calculate this change in screw if we knew to what length of DNA the observed linkage number change applied. If the full 200 base pairs of DNA in the chromatin repeat are wound superhelically in the nucleosome giving $-2\frac{1}{4}$ turns, the average screw rotation angle between bases must decrease by $(-1\frac{1}{4} + 2\frac{1}{4})/200 \times 360$ degrees ≈ 2.3 degrees. If only 140 base pairs of DNA are wound superhelically and the remaining 60 base pairs are straight as in solution, the decrease in screw angle is $(-1\frac{1}{4} + 1\frac{1}{4})/140 \times 360$ degrees ≈ 1.3 degrees. Now the DNase I cutting pattern strongly suggests that the number of base pairs per turn of DNA double helix on the nucleosome is close to 10 (36° base rotation). If so, the number of base pairs of DNA free in solution must be either 10.7 or 10.4, for these two models respectively (33.75° or 34.7° base rotation).

Now while it is commonly accepted that DNA has 10 base pairs per turn, this value comes from X-ray diffraction work on fibre patterns where the DNA is in a rather dry state and the crystal packing tends to produce an integral number of base pairs per turn. There is no reason to believe that this number must apply exactly to free DNA in solution and indeed it has been suggested³⁰ that the screw of the DNA in solution was closer to 11 base pairs per turn. The arguments are based on rather limited evidence from the position of the 12-Å peak, an experimental result which has been confirmed³¹, but which could have other explanations.

Energy calculations (M. L., in preparation) on double helical DNA show that DNA can be smoothly bent into a superhelix of radius 45 Å. The superhelical structure has a local geometry very like that of straight DNA and an energy only slightly higher than that of the straight structure. Furthermore, the straight DNA has the lowest energy with 10.7 base pairs per turn, whereas the superhelical DNA has the lowest energy with 10.0 base pairs per turn. The base pairs overlap less in the superhelically bent form to enable the base pairs to twist and tilt to accommodate the bending strain. These calculated values for the screw of straight and bent DNA are in good agreement

with the values deduced above from the combination of the results of quite different types of observation.

General features of the DNA folding

The calculations mentioned above show that it is not necessary, on merely energetic grounds, to postulate that the folding of the DNA on to the nucleosome requires sharp kinks^{32,33} at periodic intervals. Indeed, one can see *post hoc* the advantages of the nucleosome structure proposed here for folding a long, stiff molecule like DNA. It has been known for some time that DNA in solution behaves like a stiff rod (with a persistence length of 625 Å; ref. 34) but the energy calculations show that DNA can bend smoothly down to a radius of curvature of about 40 Å without undue strain (<3 kcal per 10 base pairs). Because the strain energy on bending increases as the inverse square of the radius of curvature, the superhelix should have as large a diameter as possible. One may ask then why the 140 base pairs are not merely bent into a single turn, apart from any extended protein arrangement this might require. The advantage of having more than one turn of superhelix for the DNA is that this makes it possible to have stabilising interactions between DNA in adjacent turns. The most obvious source of stabilisation would come from bridging negatively charged phosphate groups on the DNA by means of cations or positively charged histone side chains. The strength of this lateral interaction will depend on the length over which the two turns interact, the separation of adjacent turns, and the relative positions of the phosphate backbone in each turn. In the structure proposed here there are about two superhelical turns per nucleosome, which gives a full turn for lateral interactions, but still keeps the radius of curvature large. These turns are separated by about 28 Å, an ideal distance for repeated interactions between phosphate groups of double helices that are in phase. When the number of base pairs per turn of the double helix is integral (10 here) then the same stabilising interactions between adjacent superhelix turns can occur repeatedly along the chain.

How do H3 and H4 alone 'organise' the DNA?

There is a growing body of evidence^{25,35-38} that the DNA can be folded into a body with many of the features of the nucleosome core by the arginine-rich histones H3 and H4 without the intervention of the other two histones. Indeed, it was part of Kornberg's original reasoning that the arginine-rich tetramer formed the basis of the nucleosome, and that the lysine-rich histones completed the structure. The first evidence was provided by Felsenfeld and his colleagues as judged by the protection provided against micrococcal and other nucleases^{35,36} and more recently it has been demonstrated by Chambon and his colleagues²⁵ that distinct subnucleosomal particles of diameter about 80 Å can be formed by reconstitution with only the histones H3 and H4 and that these particles contain about 130 base pairs which have about the same amount of supercoiling as a complete nucleosome core containing all four histones. It also seems likely that it is only a single tetramer which is involved rather than a pair of tetramers simulating an octamer (P. Chambon, private communication; see also ref. 37).

The simplest explanation³⁵ for this sub-nucleosomal particle is that the 140 base pairs of DNA condense around a central core or kernel formed by the H3-H4 tetramer, but other more open structures are possible which take into account the stiffness of the DNA and which moreover can be regarded as incomplete nucleosome cores leading to the basic structure described here.

This may be accomplished in two ways. The first is that the H3-H4 tetramer is asymmetrically located in the

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Immunological selection of tumour cells which have lost SV40 antigen expression

P. T. Mora, C. Chang, L. Couvillion, J. M. Kuster & V. W. McFarland

Macromolecular Biology Section, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

In an already tumorigenic spontaneously transformed mouse cell, after further transformation by SV40, the virus-specific antigenic function becomes dominant. By transplantation into syngeneic mice SV40 antigen negative revertant tumour cells can be selected out.

THE nature and function of early proteins of oncogenic viruses are receiving considerable attention. For simian virus 40 (SV40) the early protein, coded by about half of the SV40 genome, has dual functions. One is apparently as a regulator protein with a crucial role in the induction of cell division, the initiation of viral DNA replication and in cell 'transformation'. The second is antigenic. In the transformed cells these two type of functions, paradoxically, may oppose each other *in vivo*. The transplantation antigen can be the cause of immunological recognition and rejection.

SV40 (virus), or cells transformed by SV40 in tissue culture are tumorigenic only in the newborn hamster and *Mastomys*. (*Rattus (Mastomys) natalensis* is an African rodent intermediate in size between the rat and the mouse.) Neither SV40 nor the cells transformed in culture by SV40 are as a rule tumorigenic in the mouse¹ or in the rat²,

or as far as it is known in any other species including the natural host of the virus the simian monkey. Tumours have been obtained in the mouse from SV40 transformed non producer cells, but only after considerable time and a number of tissue culture transfers (t.c.t. >50) followed the SV40 transformation step, and when relatively large number of cells (about 10⁶) were injected^{3–5}. Even then, most of the tumours were transitory, and were eventually rejected by the mice^{3–6}. If the immunological competence of the mouse is weak, like in the newborn, or is impaired (by, for example, irradiation, anti-lymphocyte serum, or neonatal thymectomy) SV40 T antigen-positive tumours may be produced more readily than in the immunologically competent syngeneic mouse^{4,5,7}. Such tumours then become retransplantable in the immunologically competent mouse.

In the AL/N mouse strain an SV40 transformed cell line SV AL/N, even after very large number (~275) of t.c.t. and at 10⁸ cell dose is rejected by the immunologically competent adult mice⁸. The recognition and rejection is due to SV40 specific surface and transplantation antigens^{9,10}. In irradiated mice tumours can be caused by injecting 10⁷ SV AL/N cells. The tumour cells re-established in culture retain SV40-specific nuclear (T) and cell surface antigens¹¹, and have increased tumorigenicity: 10⁴–10⁵ cells induce tumour in the immunologically competent mouse⁸.

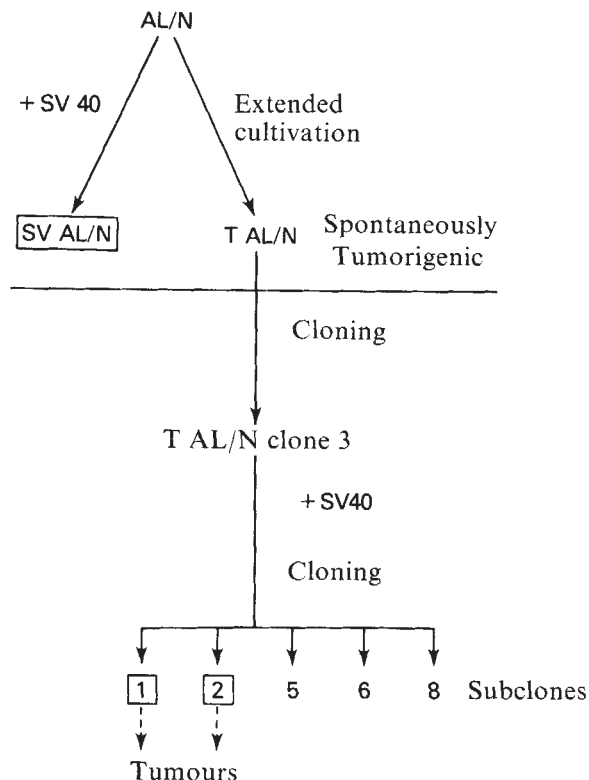


Fig. 1 Cell genealogy and cloning. The box indicates the SV40-positive cell lines. Above the horizontal lines are the mass cell lines. For cloning the T AL/N cells at t.c.t. 68 were well dispersed by trypsinisation, and 0.2 ml diluted aliquots in growth medium (reinforced Eagles with doubled amino acid and vitamins, with added antibiotics and 10% foetal calf serum) containing on the averages 0.4 cells were put into each of the 96 wells of a Falcon microtests plate. Each well was surveyed daily, and clones were picked only from wells in which there was only one cell at the onset, and the cell growth was from one focus. A single clonal line T AL/N C1 3 was selected. For infection 2.5×10^5 T AL/N clone 3 cells were exposed in 1 ml Eagles medium for 1 h at 37 °C in a shaking water bath to 9×10^7 plaque forming units (PFU) SV40, small plaque forming strain 776. This ratio of PFU per cell (360) was chosen to obtain 30%–50% T antigen positive cells at 2 d after infection. The cells were then placed in growth medium in a 10-cm Falcon Petri dish and cultivated as before¹². After t.c.t. at 5 d into two dishes and a total of 10 d growth, there were $\sim 10^7$ cells, of which 30%–40% were T-antigen positive (T antigen tests by nuclear fluorescence were carried out as previously⁸ on separate aliquots). At this time the cells were recloned using the method described above. Five subclones, each clearly grown up from one cell, were obtained. Substantially all (> 95%) of the cells of the subclones designated 1 and 2 were strongly T antigen positive when tested repeatedly up to > 200 t.c.t., while all of the cells of the subclones 5, 6, 8 were T antigen negative. Arrows with broken line indicate the origin of the T-antigen negative (revertant) tumour cell lines.

From the same AL/N cell line from which the SV AL/N cells were obtained, a highly tumorigenic spontaneously transformed T AL/N cell appeared during tissue culture. After 40 t.c.t. 10^3 T AL/N cells gave progressively growing and lethal fibrosarcomas when injected intramuscularly or subcutaneously into the adult immunologically competent syngeneic mouse^{8,9,12}.

What would happen to the tumorigenicity of the T AL/N cells if they were infected and transformed also by SV40? The SV40 induced cell surface and transplantation antigens were expected to facilitate rejection in the syngeneic mouse, opposing the tumorigenicity of the T AL/N cells. This system allowed us to embark on a novel study on the balance between strong cellular tumorigenicity and a superimposed strong and well characterisable (SV40 specific) antigenic property.

Transformation by SV40 is an inefficient process, since

only a minor fraction of the infected cells have stable insertion and thus expression of SV40 DNA. The SV40 may infect and transform cells selectively which have greater, or lower tumorigenicity. To avoid such selection we first cloned the T AL/N cells. The resulting cells, T AL/N clone 3 had retained the high tumorigenicity. After infection by SV40 of the T AL/N clone 3 cells, both T antigen-positive and negative subclones were obtained and characterised (see Fig. 1). The SV40 T antigen positive subclones were about 100-fold less tumorigenic than the T antigen-negative sister subclones or the parent T AL/N clone 3 cells. When the tumours from the T antigen positive subclones were re-established in culture, we found that SV40 T, surface and transplantation antigen negative (revertant) tumour cells resulted.

We describe here the conditions when such *in vivo* selection reproducibly occurs, prove that the revertant tumour cells are true progenies of an SV40 transformed cell, and also describe some of the phenotypic properties of the various cells. We also show that the ability of cells to grow without anchorage in a viscous medium is not a general correlate of tumorigenicity in the syngeneic mouse.

AL/N mouse cell clones

The genealogy and certain properties of the cell lines are summarised in Fig. 1 and Table 1. The AL/N mouse

Fig. 2 Presence of SV40-specific surface antigen in the T antigen-positive cells. % Chromium release represents residual cytotoxicity of an antiserum prepared in the syngeneic mouse against SV AL/N cells after the absorption by the different cells. ●, Control (no cells were used for absorption); △, absorption by T AL/N clone 3 cells; □, absorption by the T antigen-negative subclone 5 cells; ○, absorption by the T antigen-positive subclone 1 cells. Similarly, there was no significant absorption by the cells of the T antigen negative subclone 6, but almost complete absorption of the cytotoxicity by cells of the T antigen-positive subclone 2 (data not shown). For absorption 10^6 cells were plated into 60-mm Falcon plastic tissue culture Petri dishes. After 24 h growth the near confluent cell layer ($\sim 2 \times 10^6$ cells) was washed twice with the growth medium, and 400- μ l aliquots of antisera (diluted 1:200 with growth medium) were incubated (30 min, 37 °C) sequentially with three such dishes containing 2×10^6 cells. The absorbed antisera were centrifuged at 500g for 5 min, and further dilutions were tested for residual cytotoxicity on SV AL/N target cells, by a method described before^{18,19}.

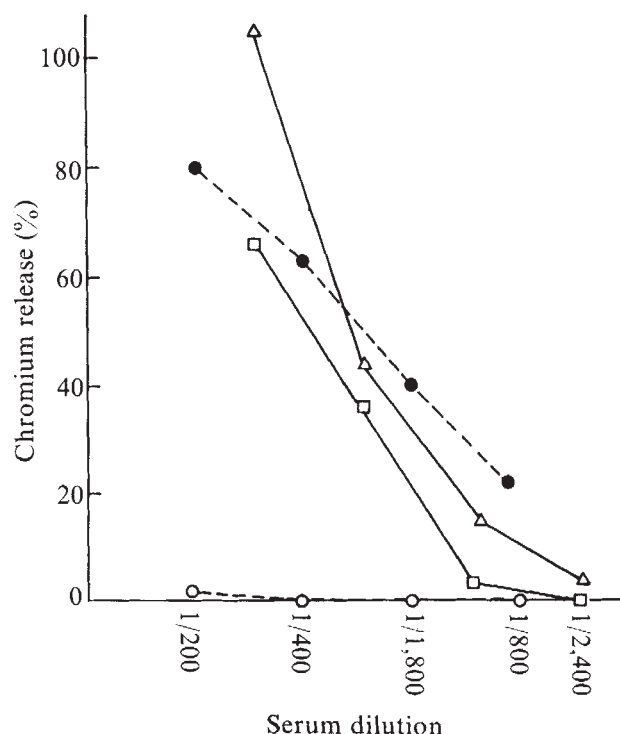


Table 1 Summary of the biological properties of the cell lines

Cell line	SV40 T antigen	Tumorigenicity TD ₅₀ * (Cells per mouse)	Time to death† (weeks)	Tissue culture growth property Saturation‡ density (cells cm ⁻² × 10 ⁶)	Colony formation in viscous Methocell suspension‡ (%)
T AL/N mass line	—	<10 ²	9	5.5	2
T AL/N clone 3	—	10 ^{1.7}	14	3.0	0.007
SV40 infected					
Subclone 1	+	10 ^{3.8}	13	1.0§	20
2	+	10 ^{3.9}	14	0.6§	10
5	—	10 ^{1.5}	17	2.4	<0.0001
6	—	<10 ¹	21	3.5	0.06
8	—	10 ^{2.5}	16	3.1	0.03
SV AL/N mass line	+	>10 ⁸	—	5.3	10

*Tumorigenicity was tested by intramuscular (i.m.) injection of cells at least in three dilutions each differing 10-fold, into groups of 6–8-week-old AL/N mice, 10 mice in each group. TD₅₀ is the cell dose which caused in 50% of the animals large (min. 1cm Ø), and eventually lethal, tumours. It was calculated as follows: $TD = 10^{d+x}$; where d is the ($\log_{10}$) number of cells which caused tumour in just below 50% of the mice, and $x = (a-50)/(a-b)$ where a is the % of mice with tumours within that group of 10 mice which had nearest to but above 50% tumours, and where b is the % nearest to but below 50%. The TD₅₀ values were reproducible within $\pm 0.5 \log_{10}$ at 10² cell dose, and $\pm 0.2 \log_{10}$ at 10⁴ cell dose.

†Time when substantially all the animals, injected (i.m.) with a cell dose rounded up to the nearest integer of the exponential of TD₅₀, die off due to progressive tumour growth. There were no regressing tumours.

‡Determined in the presence of 10% foetal calf serum⁸.

§Cells after reaching confluency tend to detach from the substratum.

embryo cell line—parent line to all AL/N cells reported here—at t.c.t. 28 had a median tumour cell dose, TD₅₀ of 10⁴–10⁵ (ref. 12); and at t.c.t. 40, TD₅₀ <10² (ref. 8). This T AL/N cell was characterised again at 65 t.c.t. (Table 1), and cloned as described in the legend to Fig. 1.

The SV AL/N cells were rejected. The spontaneously transformed T AL/N cells and the derivative T AL/N C1 3 cells were strongly tumorigenic (TD₅₀ ~10²), as were the SV40 T antigen-negative subclones 5, 6, and 9. But, the T antigen-positive subclones 1 and 2 were about 100 fold less tumorigenic (TD₅₀ ~10⁴), than the parent T AL/N C1 3 cells, or the sister subclones 5, 6, and 8 (Table 1).

The SV40 T, transplanted, and cell surface antigens probably represent modifications of products of the same SV40 cistron^{14,15}. The antigens co-purify^{10,14}, and mutants in *tsA* coordinately affect expression of all three antigens¹³. Experiment 1 in Table 2 suggests the presence of SV40 specific transplanted antigen(s) in whole subclone 1 and 2 cells; this then may be the cause of the reduced tumorigenicity of these cells. Experiments 2 and 3 in Table 2 demonstrate the presence of SV40-specific transplanted antigens in extracts of subclone 1 cells, but the absence in extracts of the T antigen-negative cells, such as subclone 5, T AL/N clone 3 cells, and a tumour cell line (number 124) obtained from subclone 1 cells (see below). Similarly, SV40-specific surface antigen was shown to be present in the T antigen-positive cells, but not in the corresponding T antigen-negative cells, when tested by an SV40-specific serum mediated cytotoxicity microassay (Fig. 2).

Cells of both of the SV40 antigen-positive subclones 1 and 2 carry in a rescuable form²⁰ the SV40 virus used for the transformation: by co-cultivation with permissive (CV-1 monkey) cells and fusion with ultraviolet light-inactivated Sendai virus, from the heterokaryons formed, infectious SV40 small plaque-forming virus was recovered: 7 × 10⁶ plaque-forming units (PFU) per ml from subclone 1, 2 × 10⁵ PFU per ml from subclone 2.

Tumour cell lines

Tumours were induced repeatedly by injecting 10⁴ or 10⁵ cells of subclones 1 and 2, into the hind leg of syngeneic adult AL/N mice, and from the tumours cell lines were established as before⁸. Chronologically at first, four tumour cell lines were obtained: first tumour line from 10⁴ subclone 1 cells at t.c.t. 17 (after the SV40 infection) designated cell

line 113; second from 10⁵ subclone 1 cells at t.c.t. 25; third from 10⁴ subclone 2 cells at t.c.t. 21; fourth from 10⁵ subclone 2 at t.c.t. 22. All four tumours were hard, encapsulated, and were identified histopathologically as fibrosarcomas, similar⁸ to those induced by the T AL/N or the T AL/N C1 3 cells. All cells from all four tumours were found completely devoid of SV40 T antigen by the nuclear immunofluorescence test⁸ in primary culture, or after additional and prolonged culturing (>30 t.c.t.). The SV40 T antigen-positive cells apparently were rejected by the immunologically competent mice, and tumours grow up only from SV40 T antigen-negative cells.

Nude mice—congenitally athymic thus deficient in thymus-dependent immunity—would not be expected to reject the SV40 T, surface and transplanted antigen-positive cells. To test this, *nude* mice (random +/nu × nu/nu, from NIH Laboratory Animal Genetic Center) were injected with 2 × 10³ subclone 1 cells, and tumours were obtained within 4 weeks in most animals (11 of 14 mice). Cells of these tumours (4 tested) were all positive for SV40 T antigen by both nuclear fluorescence and the quantitative complement fixation test, both in

Fig. 3 Tests for SV40-specific surface antigen in various cell lines. The % cell lysis is the same as % chromium release in Fig. 2, and represent residual cytotoxicity against the SV AL/N target cells, after absorption by the various cell lines. Control (no cells used for absorption): —▽—; absorption by subclone 5 cells: —□—; absorption by subclone 1 tumour cells from a syngeneic mouse (cell line 113): —△—; absorption by subclone 1 cells: —○—; absorption by subclone 1 tumour cell from a nude mouse: —□—. Absorption and assay conditions as in Fig. 2.

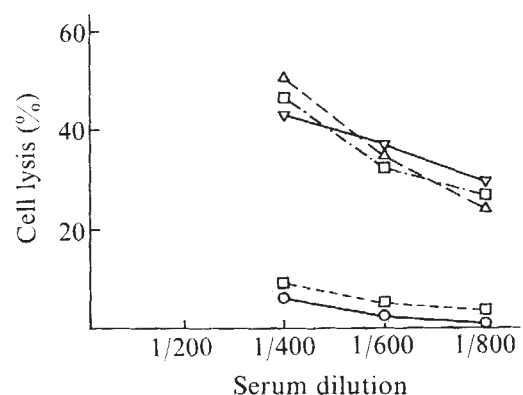


Table 2 Tests for SV40-specific transplantation antigens

Immunisation		Challenge		Tumour	<i>P</i>
Cell or extract	Dose number or mg	Cell type	Number	death/inoculated	
Experiment 1					
Subclone 1 cell	10 ³ (×4)	'M' SV AL/N T ²	10 ⁴	0/10	<0.05
	10 ³ (×4)		10 ⁵	0/10	<0.05
Subclone 2 cell	10 ³ (×4)		10 ⁴	0/10	<0.05
	10 ³ (×4)		10 ⁵	0/10	<0.05
Control	—		10 ⁴	4/10	
	—		10 ⁵	5/10	
Experiment 2a					
Subclone 1 extract	1	mKSA-ASC	10 ⁴	0/7	<0.005
	1		10 ⁵	1/8	<0.005
Subclone 5 extract	1		10 ⁴	4/7	NS
	1		10 ⁵	7/8	NS
T AL/N Clone 3 extract	1		10 ⁴	3/7	NS
	1		10 ⁵	7/8	NS
Control	—		10 ⁴	9/11	
Experiment 2b					
	—		10 ⁵	20/20	
Subclone-1 extract	1		10 ⁴	0/7	<0.005
	0.2		10 ⁴	2/7	<0.005
Control	—		10 ⁴	10/10	
Subclone 1 extract	1	Meth-A	10 ⁴	5/6	NS
	0.2		10 ⁴	5/7	NS
Control	—		10 ⁴	7/7	
Experiment 3					
Subclone 1 tumour 124 extract	1	mKSA-ASC	10 ⁴	5/5	
	0.5		10 ⁴	5/5	
	0.25		10 ⁴	5/5	
Control	—		10 ⁴	8/8	

In experiment 1 syngeneic AL/N mice were immunised by i.m. injection $\times 4$ weekly apart (into right hind leg) of the given number of subclone 1 or 2 cells. These mice were challenged 1 week after the last immunisation by SV40-positive tumorigenic cells 'M' SV AL/N T², obtained and characterised previously⁸, by injection into their left hind leg. The number of progressively growing tumours in the left hind leg is given. In experiments 2a and b BALB/c mice were immunised by extracts (mg protein) of the various cells obtained with 0.5% Triton X-100 (refs 10, 16). The mice were challenged by an SV40-transformed BALB/c mouse kidney cell mKSA (ref. 3) which after repeated transplantation in the BALB/c mice⁹ was established in ascites form; mKSA-ASC¹⁷; it was shown before that cells in both forms contain SV40-specific transplantation antigen^{3,17}. For specificity control the challenge was by a chemically transformed Meth A BALB/c mouse embryo cell. Tumour deaths occurred by ascites or by fibrosarcoma at the site of challenge after about 1 month, groups were observed for 3 months. Experiment 3 shows the absence of SV40-specific transplantation antigen in the extract of tumour cells 124 (see text); these experiments were done similar to, and simultaneously with experiment 2. Control mice were injected with TBS. P values were calculated as before¹⁴; NS, not significant.

primary culture and also after various numbers (~ 10) of tissue culture transfers.

When tested for SV40-specific surface antigen, all tumour cell lines obtained from the syngeneic animals were devoid, but the tumour cells from the nude mice possessed this surface antigen. Examples, with controls, are given in Fig. 3; clearly, subclone 1 tumour cells (compare line 113) do not possess SV40-specific surface antigen. The subclone 1 tumour cell lines (for example 124, see below) were devoid of SV40-specific transplantation antigen (Table 2, experiment 3).

There are two possibilities to explain the *in vivo* selection of SV40 antigen negative tumour cells. The first possibility is that a T antigen-positive subclone, say subclone 1, is not a true subclone, but is consistently contaminated with a low number of (about 10^{-3}) of untransformed T AL/N clone 3 cells (which cannot be detected even by the T antigen nuclear staining) and which can grow out as tumours; while the majority of the cells which are the SV40-specific T, surface and transplantation antigen-positive cells are all rejected by the immunocompetent mouse. The second possibility is that subclone 1 was indeed a good clone, free of T AL/N clone 3 cells, but a certain small (~ 1 – 0.1%) fraction or number of subclone 1 cells loses (the ability to express) SV40 antigens, and these revertant cells are then selected out to grow as tumours, while the SV40 T, surface, and transplantation antigen-positive cells, which are in great (about 10^2 – 10^3) excess, are all rejected.

Recloning of subclone 1 cells gave only T antigen-positive cells (six reclones tested). Co-cultivation experiments of known number of subclone 1 and T AL/N clone 3 cells

indicated no preferential growth of subclone 1 cells (data not shown). Both of these made it unlikely that subclone 1 had originated from the two type of cells. It took, however, a fortunate finding of characteristic marker chromosome translocation to virtually eliminate the first possibility. When chromosomes were banded from subclone 1 cells and from the derivative tumour cells 113, three common marker chromosomes, number 1, 2 and 3 in Fig. 4, were found clearly in each metaphase of these two cell lines. Metaphases were chosen at random and 17 metaphases from subclone 1, and 14 metaphases in the resulting tumour cells 113 were compared. However, when banding the T AL/N clone 3 cells (at t.c.t. 21, chromosome modality 38), in 20 randomly chosen metaphases none was found to possess the easily identifiable chromosomal marker 1, but clearly all were found to possess the easily identifiable chromosomal markers (classified as Robertsonian translocations) proving that the subclone 1 cells but not the T AL/N clone 3 cells are the direct parents of the tumour cells.

Being assured that subclone 1 is indeed a true clone, we used the subclone 1 cells to check the reproducibility of selecting SV40 antigen negative tumour cell lines by *in vivo* passage through the immunologically competent syngeneic mouse. At t.c.t. 12, 10^4 subclone 1 cells repeatedly induced T and surface antigen negative tumour cell lines (see Table 3, tumour cell lines 127 and 128), as did 10^5 subclone 1 cells (cell line 124). Six cloned lines were obtained from tumour cell line 124, these were designated as cell lines 129–134, and all were found to be T antigen negative. The tumorigenicity of tumour cells 124 was high ($TD_{50} = 10^2$), similar to other T antigen negative cells;

however, its clonal derivatives 129–134 had various TD_{50} values between 10^2 and $>10^4$, indicating that cloning may select for differences in tumorigenicity. These and other phenotypic data are summarised in Table 3.

Thus, the true clonal subclone 1 cells give reproducibly SV40 antigen negative tumours when passed through the immunologically competent mice. These tumours can be obtained after injection of as few as 10^4 or 10^5 cells. The tumour cells can possess high tumorigenicity ($TD_{50} \sim 10^2$) and the resulting fibrosarcomas never recess: the tumour cells in this respect are similar to the T AL/N C1 3 parent cells or to the T antigen-negative sister subclones. Apparently subclone 1 cells are prone to revert or segregate at a high frequency to a stably SV40 antigen negative cell line. Current studies by DNA reassociation kinetics indicate that subclone 1 cells contain approximately 1 genome equivalent of SV40 DNA, while the tumour lines or tumour clones tested contain about 0.5 copies per diploid complement of DNA which seem to be depleted in some of the early half of the SV40 genome, and which are not transcribed to RNA. These observations (J.M.K., P.T.M., M. Brown and G. Khoury, in the press) are consistent with the biological and chromosome findings.

The tumorigenicity in the syngeneic host of the cell lines investigated does not correlate with anchorage-dependent growth in viscous medium. There were highly tumorigenic cells with negligible or no observable growth in

Fig. 4 Marker chromosomes. Partial karyotypes of four subclone 1 cells (banded at t.c.t. 22) and four resulting tumour cell 113 metaphases (banded after 12 t.c.t. following the re-establishment) are compared pairwise in rows a, b, c, d. The three marker chromosomes present in these cells are arranged columns 1, 2 and 3. Chromosome(s) from the subclone 1 cells appear in left side of the columns. Chromosome(s) from the resulting tumour cells 113 appear in right side of column. Slides were stained with quinacrine-mustard for Q banding²¹, and photographed with a Zeiss photomicroscope III equipped for epi-fluorescent microscopy. After photomicrographic enlargements of the chromosomes, karyotypes were numbered and arranged according to size²². The subclone 1 cells were essential sub-diploid (chromosome modality 36) at t.c.t. 16, but some cells became polyploid when banded (t.c.t. 22) which explains why some of the marker chromosomes are present more than once. Tumour cells 113 were essentially sub-diploid (chromosome modality 33) at t.c.t. 8 and 12.

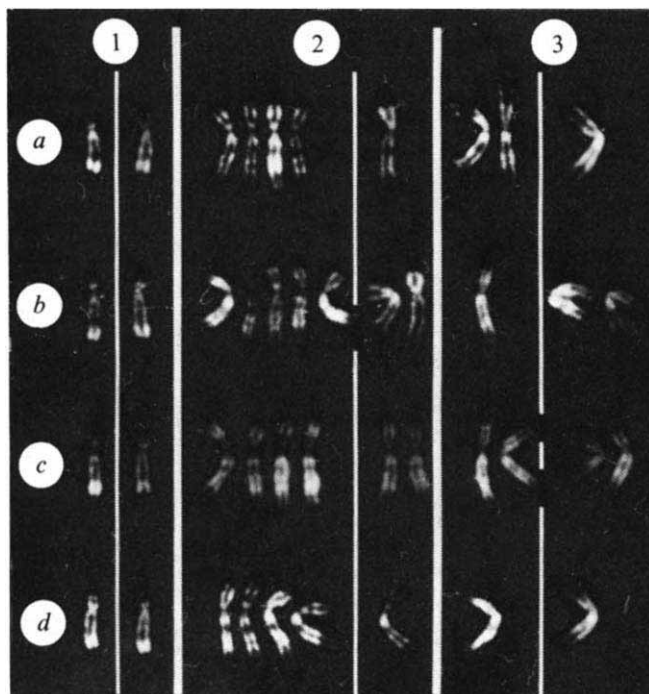


Table 3 Properties of the subclone 1 tumour cells and of clones of tumour line 124

Tumour cell line	Tumorigenicity (TD_{50})	Colony formation in viscous Methocell suspension (%)
113	NT	10
124	10^2	0.18
127	NT	0
128	NT	0.01
Clones of 124		
129	10^2	0.01
130	10^2	0.28
131	10^3	0
132	$>10^4$	1.5
133	10^3	0.12
134	$>10^4$	0

All tumour cells were SV40 T and surface antigen negative; all the clones of 124 were tested only for SV40 T antigen, they were all negative. The tumour line 124 was cloned by a single cell technique. Tumorigenicity and colony formation in viscous Methocell were determined as in Table 1. NT, not tested.

Methocell (see Tables 1, 3). We have similar observations on other BALB/c and AL/N highly tumorigenic cloned cells (data not shown). Neither did the presence and functioning of the SV40 early gene correlated absolutely with anchorage-independent growth: the tumour cell line 113 is SV40 antigen negative, but grows about as well in Methocell as the SV40 antigen-positive subclones 1 and 2. A recent publication appeared with partially contrasting claims in other less well matched cell systems²³.

The above experiments open up new kinds of genetic, immunochemical and biochemical studies using the strong *in vivo* antigen activity endowed to a mammalian tumour cell by transformation with SV40 to cause rejection. It also gives a novel perspective on biological consequences of SV40 transformation of cells, probably in the majority of the mammalian species. We emphasise, however, that the above experiments show that SV40 endows immunogenicity to, and allows recognition and rejection of, only those tumorigenic cells which were transformed by SV40, and may not alter the tumour-specific transplantation antigen associated with the spontaneously occurring or otherwise induced malignant transformation.

We thank Miss Cecily Sun for chromosomal banding, Drs O. J. and D. A. Miller for review of the marker chromosomes, and Dr L. W. Law for the ascites cell line.

Note added in proof: The revertant tumour cells (cf. clone 130) can be re-transformed by SV40, and on transplantation of a T antigen-positive clone T antigen-negative revertant tumour cell line can be obtained again.

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letters to nature

Upper limits for the radio pulse emission rate from exploding black holes

It has been suggested¹ that the explosion of a primordial black hole (pbh) might produce a coherent radio pulse detectable at a distance of $\sim 10^4$ pc with a simple antenna. Several searches²⁻⁵ for radio pulses arriving from random directions in space, such as might be produced by supernovae⁵⁻⁷, have already been carried out using wide beam (all-sky) antennas, and the negative results obtained are used here to place limits on the pbh radio pulse emission rate per unit volume. An indication has already been given⁸ of the limits which some previous radio searches impose, and of the influence of antenna aperture and beamwidth on achievable limits. This paper presents a more general analysis of limit criteria, including consideration of the effects on the pulse timescale of the interstellar medium, and examines the factors which determine the optimum search strategy for pbh radio pulses. It is reported that the best radio limit⁴ known to the author, could be improved by at least times 10^6 using existing techniques. In addition, examination of this existing limit⁴ shows that it is already ~ 80 times better (99.5% confidence level) than a limit which it has been suggested⁸ might be attainable in the optical region, and is $\sim 10^6$ times better than pbh explosion rate limits obtained from γ -ray observations^{9,10}, although the γ -ray limits do not involve the generation of a coherent electromagnetic pulse.

We assume that the pbh's are concentrated in galactic haloes (radii $\simeq 20$ kpc)⁹ and that the pulse energy, E_r , in J (ref. 1) is given by

$$E_r \simeq 1.5 \times 10^{28} \nu^{-0.33} \quad (1)$$

where ν is the characteristic frequency (Hz) of emission. The value of the characteristic frequency is highly uncertain¹, and so the situation is considered where ν is equal to the frequency of observation. The attainable limit, L (volume⁻³ time⁻¹), established by a negative search at the 99.5% confidence level is given by

$$L \simeq 5 (VS)^{-1} \quad (2)$$

where S is the duration of the search. V , the volume of space observed above the threshold sensitivity, is given by

$$V \simeq (\Omega/3) R_M^3 \quad (3)$$

where Ω is the antenna beam solid angle and R_M is the maximum distance at which detection is possible. We adopt a detection threshold of 5σ signal-to-noise ratio and examine the factors which determine R_M . For an antenna of gain g (relative to an isotropic radiator), and receiver bandwidth $\Delta\nu$, the pulse energy collected (E_{col}) is at least

$$E_{\text{col}} \simeq \frac{1}{4} (E_r / 4\pi R_M^2) (\Delta\nu/\nu) (gc^2/4\pi\nu^2) \quad (4)$$

$$= 2 \times 10^{42} \nu^{-3.33} \Delta\nu R_M^{-2} g \quad \text{J} \quad (5)$$

where c is the velocity of light and R_M is in metres. (The factor of $\frac{1}{4}$ allows for the source being up to -3 dB away from the beam axis ($\frac{1}{2}$) and for only one polarisation being observed ($\frac{1}{2}$)).

The minimum pulse energy, E_{min} , which the radiometer can detect, is

$$E_{\text{min}} \simeq 5kT(\Delta\nu\tau)^{0.5} \quad (6)$$

(where k is Boltzmann's constant, T is the system temperature, and τ is the integration time), provided that the pulse duration at the integrator input, $t_p \leq \tau$. The intrinsic timescale of the pulse is $\sim 1/\nu$ (ref. 1), so that t_p is principally determined by the radiometer response time $\simeq 1/\Delta\nu$, by interstellar dispersion broadening over the band $\Delta\nu$, and by interstellar scattering. If $\Delta\nu$ is gradually increased, the signal-to-noise ratio will improve until the pulse time scale is dominated by dispersion. Once this stage is reached ($\Delta\nu \simeq 10^4 - 10^5$ Hz for typical dispersion measures) little further improvement in signal-to-noise ratio is obtainable by further increase in $\Delta\nu$ (see equation (9) below), unless de-dispersion is used. The timescale, t_p , in s due to dispersion is given by

$$t_D = 2.8 \times 10^{-7} \nu^{-3} \Delta\nu DM \quad (7)$$

where DM is the dispersion measure in m^{-2} . Setting $t = t_D = \tau$ in equation (6) above gives

$$E_{\text{min}} \simeq 4 \times 10^{-26} T \nu^{-1.5} \Delta\nu (DM)^{0.5} \quad \text{J} \quad (8)$$

where T is in Kelvins. Equating E_{col} and E_{min} , gives

$$R_M \simeq 8 \times 10^{33} \nu^{-0.92} T^{-0.5} g^{0.5} (DM)^{-0.25} \quad \text{m} \quad (9)$$

$\Delta\nu$ has been eliminated from this expression.

In general, the system temperature, T , is a function of both sky noise and receiver noise. To minimise T (and DM), observations should be directed towards the regions of the galactic poles, where (for the north galactic pole (NGP)) the sky temperature, T_{sky} , is given by¹¹⁻¹³

$$T_{\text{sky}} \simeq 2 \times 10^{24} \nu^{-2.7} \quad (10)$$

This gives $T_{\text{sky}} \simeq 80$ K at 200 MHz. Above this frequency, receiver noise will begin to dominate the system noise, so that further increase in ν will tend to reduce sensitivity. Therefore, for observations near the NGP at $\nu \simeq 200$ MHz with a sky noise limited system, equation (9) becomes

$$R_M \simeq 5 \times 10^{21} \nu^{0.43} g^{0.5} (DM)^{-0.25} \quad (11)$$

To estimate DM , a model is adopted¹⁴ in which the electron distribution is uniformly stratified parallel to the galactic plane and has an exponential distribution perpendicular to the plane

with a scale height of 600 pc (refs 14, 15) and a density at the plane of $3.3 \times 10^4 \text{ m}^{-3}$. Thus, DM is given in m^{-2} by

$$DM \simeq 6 \times 10^{23} (1 - \exp(-R_M \sin b / 1.8 \times 10^{19})) / \sin b \quad (12)$$

where b is the galactic latitude. If $g \gtrsim 10$, the antenna beam limits (-3 dB) will be less than 30° from the NGP so that to a good approximation, $\sin b \simeq 1$. For $g = 10$ (a small, simple antenna) and $\nu = 200 \text{ MHz}$, equations (11) and (12) give $R_M \simeq 2.2 \text{ kpc}$. Putting the expression for R_M given by equation (11) into equation (3) gives as the volume of space observed

$$V \simeq 7 \times 10^{65} \nu^{1.3} g^{0.5} (DM)^{-0.75} \quad \text{m}^3 \quad (13)$$

which, for $g = 10$ and $\nu = 200 \text{ MHz}$, is $6 \times 10^9 \text{ pc}^3$. Thus, observation for one year (assuming no pulses were detected) could yield a 99.5% confidence limit of $\sim 10^{-9} \text{ pc}^{-3} \text{ yr}^{-1}$. Use of an antenna 10 times larger in diameter ($\sim 15 \text{ m}$ at 200 MHz) for the same observation period might improve this limit 10-fold and would observe to the edge of the halo. The long observation times required for further limit improvement may rule out the use of still larger antennas. An Arecibo-type antenna, however, using existing de-dispersion facilities might just reach out to $\sim 700 \text{ kpc}$ in the approximate direction of the NGP, but to be worthwhile, the increased volume would have to contain an external galaxy. A more effective way of improving the limit would be to use a multiply-phased array. A 10×10 element array at 200 MHz could observe to the halo edge, and still maintain a $\sim 1\text{-sr}$ solid angle, thereby offering a potential 1,000-fold increase in the volume of space observed, relative to a single element ($g = 10$), and so attain a limit of $\sim 10^{-12} \text{ pc}^{-3} \text{ yr}^{-1}$ in 1 yr observation.

The above results for R_M and V are obtained assuming a rather idealised situation in which the characteristic emission frequency is $200 \text{ MHz} \simeq$ observation frequency, and observations directed towards the NGP are carried out with a sky noise limited system and correctly optimised integration time. For this situation, and with $g \gtrsim 10$, $R_M \gtrsim 4 \times$ scale height of the electron distribution, so that to a good approximation DM is independent of R_M and from equation (13)

$$V \propto g^{0.5} \quad (14)$$

Previous all-sky searches, however, included observations both near to and far from the NGP, and were generally not sky noise limited and did not have optimised integration times. The effect was that these searches were usually confined within the galactic plasma, so that DM was no longer approximately independent of R_M (equation (12)) and the proportionality in equation (14) was not necessarily valid. For any particular search

$$R_M = (E_t / (4\pi W \nu t_i))^{0.5} \quad (15)$$

where W is the search flux threshold per unit bandwidth, and the output pulse timescale, t_i , is determined by the dispersion, scattering, bandwidth and integration time which the pulse encounters. In all previous searches, $t_p \simeq t_D$, so that when $\tau \gg t_p$, $t_i \simeq \tau$, and putting the expression for R_M given by equation (15) into equation (3) shows that proportionality (14) is applicable in this case. When $\tau \lesssim t_D$, V must be obtained, however, by solving equations (12) and (15) for R_M and then substituting the expression obtained into equation (3). The result is that when $\tau \lesssim t_D$, V is a weaker function of g than it is in equation (14) and in the extreme case where approximately $DM \propto R_M$ (for example, when observing

along the galactic plane) and $\tau \ll t_D$, V is independent of g . Thus, it is important to observe well out of the galactic plane and have $\tau \simeq t_D$.

Table 1 gives the best pbh radio pulse rate limits I know (99.5% confidence level) within the frequency decades 10–100 MHz, 100 MHz–1 GHz, and 1–10 GHz, and the actual frequencies at which they were obtained. In each case, it is assumed that the characteristic emission frequency equals the observation frequency. Also shown are the corresponding values of R_M for each

Table 1 Primordial black hole radio pulse rate limits

Reference	Frequency range (MHz)	Rate limit ($\text{pc}^{-3} \text{ yr}^{-1}$)	Frequency (MHz)	Distance, R_M (pc)
*	10–100	1.5×10^{-6}	45	370
4	100–1,000	5×10^{-7}	270	975
5	1,000–10,000	4×10^{-5}	1,400	525

*Unpublished observations at 45 MHz, December 1970 to March 1971, carried out by R. W. P. Drever and W. P. S. Meikle (Glasgow), G. A. Baird, T. Delaney and B. G. Lawless (Dublin), R. A. Porter and R. E. Spencer (Jodrell Bank).

search. The searches at 45 MHz and 1.4 GHz had $\tau < t_D$, and the 270-MHz search had $\tau > t_D$. These limits are 10^4 – 10^6 times better than the pbh explosion rate limits achieved from γ -ray studies^{9,10}.

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W. P. S. MEIKLE

Astronomy Group,
Physics Department,
Imperial College of Science and Technology,
London, UK

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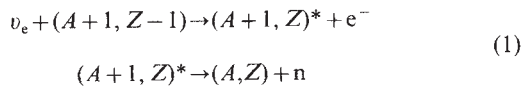
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Neutrino-induced nucleosynthesis and deuterium

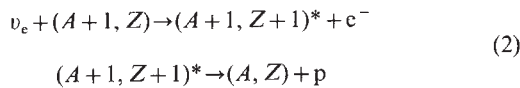
DOMOGATSKY and Nadyozhin¹ have suggested that the neutrino flux from a collapsing stellar core might induce nuclear transformation in the mantle and envelope of the surrounding pre-supernova star. They further suggest that such weak interactions might be responsible for producing the so called 'p-process' nuclei which are the rare heavy elements bypassed by the neutron capture chains responsible for synthesising most nuclei heavier than iron. This letter points out a weakness in their model as a mechanism for p-nucleosynthesis and concurrently suggests a rather novel method for the synthesis of deuterium that may have been operative during an early generation of supermassive stars ($M \sim 10^6$ – $10^8 M_\odot$).

First, consider the conditions requisite for producing p-process nuclei from the more abundant neighbouring r- and s-process isotopes (see ref. 2 for an explanation of this nomenclature).

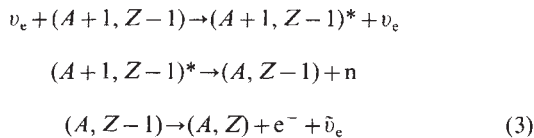
One might invoke the charged-current processes suggested by Domogatsky and Nadozhin for producing the nucleus (A, Z)



where $(A+1, Z)^*$ is a neutron unbound state of the nucleus having atomic mass $A+1$ and Z protons or by



In addition to the charged-current processes that they suggest, one might also add the neutral current process



and others that come to mind. The cross section, however, for each of these processes is very small, Domogatsky and Nadozhin estimate a value $\sigma_\nu \sim 10^{-41} \text{ cm}^2$ as typical. According to Cameron³ the relative abundance of p -nuclei to neighbouring nuclei produced by neutron capture is about 1%. Therefore, the production factor

$$P = \phi_\nu \sigma_\nu \gtrsim 0.01 \quad (4)$$

where

$$\phi_\nu = \frac{E_{\nu(\text{tot})}}{4\pi R^2 E_\nu} \quad (5)$$

R is the radius from the collapsing, neutrino-emitting core to the mass shell where the neutrino-induced nucleosynthesis occurs, and E_ν is the mean energy of the emitted neutrinos. An upper bound to the total energy emitted in neutrinos is the binding energy of a typical neutron star, 10^{53} erg. Actually a smaller number, say 10^{52} erg, is probably more appropriate⁴. Using $E_\nu = 10^{52}$ erg and $E_\nu = 15 \text{ MeV}$ one finds from equation (4) that $R \lesssim 2 \times 10^8 \text{ cm}$.

Problems arise when the conditions extant at $R = 2 \times 10^8 \text{ cm}$ in a realistic pre-supernova star^{5,6,7} are examined. For most massive pre-supernova stars of interest (say $M \gtrsim 15 M_\odot$) this region lies interior to the neon burning shell and probably interior to the oxygen burning shell and is characterised by temperatures in excess of $2 \times 10^9 \text{ K}$. At such high temperatures, all heavy seed nuclei will be rapidly photodisintegrated to iron-group nuclei. It can be estimated, using rates given by Woosley and Howard⁸, that at $T = 2 \times 10^9 \text{ K}$ all seed nuclei heavier than $A = 150$ will be destroyed by photodisintegration reactions in a time of only a few thousand seconds. This is considerably less than the time occupied by oxygen and silicon burning in the core of the star, hence seed nuclei with $A \geq 150$ are not present at $R = 2 \times 10^8 \text{ cm}$ when the star blows up. Actually the problem is even more severe than so far described. The number 0.01 used in equation (4) is applicable only if all the r - and s -process nuclei ever produced have been exposed to precisely the conditions described. Since in reality the fraction of seed nuclei so exposed has been very small the required production ratio must be correspondingly larger, $P = 0.1$, for example, if only

10% has been irradiated. Thus the required exposure radius is smaller, the temperature higher, and the likelihood of seed survival smaller. The p -process nuclei can, on the other hand, be produced by more traditional strong and electromagnetic processes that occur farther out in the star as has been recently shown by Woosley and Howard⁸.

Consider now another possibility for neutrino-induced nucleosynthesis. The reaction



can produce a neutron flux in the hydrogen envelope surrounding a strong anti-neutrino source. It is important to note that anti-neutrinos are required for this process. Neutrinos, as result from electron capture and positron decay, for example, will not work. If the density of the hydrogen exceeds roughly

$$\gtrsim 10^{-8} \left(\frac{1 \text{ mb}}{\sigma_{ny}} \right) \left(\frac{10^8 \text{ cm s}^{-1}}{v_T} \right) \text{ g cm}^{-3} \quad (7)$$

these neutrons will be captured before decaying. Here v_T is the mean velocity of the neutron and σ_{ny} is the radiative capture cross section in millibarns. It is assumed here that inelastic scattering will reduce the neutron energy to some relatively low value ($\sim \text{keV}$) before capture occurs. Even though heavy elements such as ^{56}Fe have substantially larger neutron capture cross sections than hydrogen it is assumed that the numerical preponderance of H-nuclei will make them more likely candidates for capturing the neutrons produced by equation (6). Thus deuterium is formed by



The viability of this mechanism for deuterium production depends upon (1) the existence of a sufficient anti-neutrino flux to produce D/H of at least 2×10^{-5} (ref. 9), the currently accepted interstellar value, and (2) the material irradiated being cool enough so that the deuterium formed is not destroyed by ${}^2\text{D}(p, \gamma){}^3\text{He}$ before ejection. First I shall show that supernova probably cannot satisfy these requirements.

The anti-neutrino flux from a supernova explosion is thermally produced and is not as large as the neutrino flux which receives a big contribution from electron capture. Assume the collapsing core emits 10^{52} erg of 15 MeV anti-neutrinos (this would be an upper bound). The cross section for the process in equation (6) at $E_\nu = 15 \text{ MeV}$ can be calculated¹⁰ to be roughly $2 \times 10^{-41} \text{ cm}^2$. From equations (4) and (5) the production ratio at a radius R will then be

$$P = 7 \times 10^{-6} (10^{10} \text{ cm}/R)^2$$

Since the radius of the hydrogen burning shell in a highly evolved massive star⁷ exceeds $2 \times 10^{10} \text{ cm}$ one cannot produce sufficient quantities of deuterium to account for present abundance determinations. This argument is strengthened by the fact that the hydrogen temperature does not drop below 10^7 K until $R \gtrsim 10^{11} \text{ cm}$ thus deuterium formed interior to this radius would be destroyed in less than 1,000 s (ref. 11) (less than the speed of sound communication time to the core).

I will end this letter, however, with a speculation on a site where equation (6) might function with greater efficiency. Some models for active galactic nuclei and quasars suggest the existence early in a galaxy's lifetime of supermassive stars and/or black holes¹². The explosion of a $10^8 M_\odot$ star might produce 10^{60} erg (ref. 13). The formation of a $10^6 M_\odot$ black hole would emit a similar quantity of

energy. If the collapse of these objects produces matter at very high temperature ($T \gtrsim 10^{10}$ K) a substantial fraction of this energy might be emitted as anti-neutrinos. If one takes 10^{59} erg, $E_\nu = 15$ MeV, and $R = 10^{12}$ cm, for example, a production ratio $D/H \sim 7 \times 10^{-3}$ could be attained. Even if a small fraction of all the hydrogen in a galaxy were processed through these conditions the present abundance of deuterium could be understood.

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S. E. WOOSLEY

Lick Observatory,
Board of Studies in Astronomy and Astrophysics,
University of California, Santa Cruz 95064

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Support for the astronomical theory of climatic change

AMONG recent papers supporting the astronomical theory of climatic change¹⁻⁵, the work of Hays *et al.*⁵ is of particular importance in providing detailed quasi-periods from deep-sea cores. I wish to draw attention here to the fact that the periods found are very close to the periods predicted by Berger^{6,7} in the latest and most accurate calculation of the variations of the various 'Milankovitch' parameters. The roughly 24,000- and 19,500-yr periods from the core samples are not significantly different from the periods associated with the largest amplitude terms in the series expansion of the precessional parameter $e \sin \tilde{\omega}$; the periods around 42,000 yr are essentially identical with the most important term in the expansion of the obliquity ε ; and the peaks in the range of 106,000 yr containing most of the variance, might be regarded as either a contribution from the eccentricity, where a weighted mean of the main amplitude terms has a period of 110,753 yr, or as a beat effect of precessional periods, as has been noted by Wigley⁸.

The astronomical theory of climatic change, originating from Croll⁹ and developed by Milankovitch¹⁰ has been widely discussed and needs no elaboration here. To establish the validity of

Table 1 Main terms in the series expansion of the precession parameter $e \sin \tilde{\omega}$

	Amplitude, P_i	Mean rate, α_i (" per yr)	Period (yr)	Phase, β_i (deg)
1	0.018 608 0	54.646 478	23,716	32.0
2	0.016 275 2	57.785 364	22,428	197.2
3	-0.013 006 6	68.296 536	18,976	311.7
4	0.009 888 3	67.659 819	19,155	323.6
5	-0.003 367 0	67.286 006	19,261	282.8
6	0.003 330 8	55.638 352	23,293	90.6
7	-0.002 354 0	68.670 349	18,873	352.5
8	0.001 400 2	76.656 031	16,907	131.8
9	0.001 007 0	56.798 442	22,818	157.5

Mean period over the previous 5 Myr: 21,700 yr.

that theory, the following three criteria must be met. (1) Are the quasi-periodicities of the Earth's orbital elements significantly present in the geological record?¹¹ (2) Is there any significant correlation between insolation curves and geological data?¹² (3) Can these insolation changes be correlated with climatic changes^{13,14}?

I have previously^{6,7} demonstrated the accuracy of a new astronomical solution for the long-term variations of the Earth's orbital elements. From theoretical formulae given in refs 6 and 7 it is possible to write, in an easily applicable form, the series expansion of astronomical elements required in the computation of the insolation, that is, the eccentricity e of the Earth's orbit, the precessional term $e \sin \tilde{\omega}$ ($\tilde{\omega}$ being the longitude of the perihelion measured from the moving vernal equinox) and the obliquity ε (inclination of the equator on the ecliptic):

$$\begin{aligned} e &= e_0 + \sum E_i \cos(\lambda_i t + \phi_i) \\ e \sin \tilde{\omega} &= \sum P_i \sin(\alpha_i t + \beta_i) \\ \varepsilon &= \bar{\varepsilon} + \sum A_i \cos(\gamma_i t + \delta_i) \end{aligned}$$

where $e_0 = 0.028\,706\,9$, $\bar{\varepsilon} = 23^\circ.320\,556$ and time $t = 0$ refers to 1950 AD.

Trigonometric series of this type not only make e , $\tilde{\omega}$ and ε more easily computable, but also provide us directly with a power spectral analysis of their time variation. As in this paper, the main interest lies in the spectrum of the astronomical elements, the period and mean rate associated with the only main largest amplitude terms have been reproduced in Tables 1-3. The computation of e , $e \sin \tilde{\omega}$ and ε will be detailed, and all terms of the trigonometrical expansions will be listed elsewhere.

Table 2 Main terms in the series expansion of the obliquity ε

	Amplitude, A_i	Mean rate, γ_i (" per yr)	Period (yr)	Phase, δ_i (deg)
1	-2,462.2	31.609 974	41,000	251.9
2	-857.3	32.620 504	39,730	280.8
3	-629.3	24.172 203	53,615	128.3
4	-414.3	31.983 787	40,521	292.7
5	-311.8	44.828 336	28,910	15.4
6	308.9	30.973 257	41,843	263.8
7	-162.5	43.668 246	29,678	308.4
8	-116.1	32.246 691	40,190	240.0
9	101.1	30.599 444	42,354	223.0
10	-67.7	42.681 324	30,365	268.8

Mean period over 5 Myr, 41,000 yr.

Taking the precessional component, $e \sin \tilde{\omega}$, the four largest terms have periods of 23,716, 22,428, 18,976 and 19,155 yr. The first two and the last two clearly correspond respectively to the 24,000- and 19,500-yr periods found by Hays *et al.* in deep-sea cores, with the splitting of the theoretical mean period 21,700 yr in two peaks, depending on the resolution of their spectral analysis.

In the obliquity term, not only the dominant component has a period of 41,000 yr but also 5 of the next 10 largest components have periods very close to 41,000 yr. That explains quite well the steadiness of the quasi-periodicity observed in the long-term variations of ε : the mean period over the past 5 Myr is 41,000 yr and over the next 1 Myr it will be 41,040 yr.

For the eccentricity, the situation is still more complicated. As suggested in Berger⁷, the most important term has a periodicity of 412,085 yr—too long to be clear in the analysis of Hays *et al.*—and the convergence of the series is very slow. Because of this, while the weighted mean periodicity for terms 2 to 5 in the expansion is 111,000 yr, the average period from the computed values of e is 95,800 yr, during the past 5 Myr; the prediction for the next 1 Myr being a mean of 91,400 yr. If longer cores can be investigated by

techniques allowing high resolution spectrum, I would expect the 100,000-yr peak to be split into peaks at 95,000 and 123,000 yr, with the main period of 413,000 yr becoming strongly displayed. These 95,000- and 123,000-yr periods are related either to a beat respectively between terms 1 and 3 and between terms 2 and 3 of the $e \sin \tilde{\omega}$ series expansion (Table 1), and to terms numbered 2 and 3 in series expansion of the eccentricity (Table 3).

Table 3 Main terms in the series expansion of the eccentricity e

	Amplitude, E_i	Mean rate, λ_i (" per yr)	Period (yr)	Phase, ϕ_i (deg)
1	0.011 029 40	3.138 886	412,885	165.2
2	-0.008 732 96	13.650 058	94,945	279.7
3	-0.007 492 55	10.511 172	123,297	114.5
4	0.006 723 94	13.013 341	99,590	291.6
5	0.005 812 29	9.874 455	131,248	126.4
6	-0.004 700 66	0.636 717	2,035,441	348.1
7	-0.002 544 64	12.639 528	102,535	250.8
8	0.002 314 85	0.991 874	1,306,618	58.6
9	-0.002 219 55	9.500 642	136,412	85.6
10	0.002 018 68	2.147 012	603,630	106.6
11	-0.001 723 71	0.373 813	3,466,974	40.8
12	-0.001 661 12	12.658 184	102,384	221.1
13	0.001 313 42	12.021 467	107,807	233.0

Mean period over the previous 5 Myr, 95,800 yr.

This prediction is in line with Table 4 of Hays *et al.*⁵ and also with the evidence from their Fig. 9 which suggests a positive correlation between summer temperature and eccentricity during the past 500,000 yr. Indeed, looking for the influence of e alone on the insolation, we can see that it acts only through the $(1-e^2)^{-1/2}$ factor in the total energy received by the Earth¹⁵. As e oscillated between 0.000483 and 0.060791 in the past 5 Myr, it is easily found that the total variation in solar radiation received by the Earth as compared with the present value, is no more than -0.17% or $+0.014\%$. These variations are small, but the are in the right direction according to the results of Hays *et al.*: they are positively correlated with the change in summer temperature and not in the opposite sense, as required by Milankovitch¹⁰, who stated that the influence of e was almost exclusively through the $e \sin \tilde{\omega}$ term. As a consequence, from an astronomical point of view, the 100,000-yr period seems to be closely related either to the nonlinear interaction between the two precession peaks and to the eccentricity period itself if Fig. 9 of ref. 5 can be confirmed.

Some important conclusions may be drawn from the fact that the obliquity peak in geological records is smaller than the $\sim 105,000$ -yr peak or, at least, less dominant than formerly thought¹⁶. Analysing the solar radiation available on the assumption of a perfectly transparent atmosphere¹⁷, it can be deduced that, on the one hand: (1) The insolation received at the equinoxes and the differences between the length of the summer and of the winter astronomical seasons, are functions only of the precessional parameter, $e \sin \tilde{\omega}$. And (2), at the solstices, this parameter has a larger influence than the obliquity. On the other hand: (1) During astronomical seasons, the insolation received at any latitude is a function of the obliquity ϵ . And (2), at high latitudes, the deviations of the insolation from present day values for the caloric seasons are mostly functions of ϵ (ref. 18).

Thus, as the 'eccentricity-precessional beat' period seems to have an important role in past climates as recorded in geological data, the seasonal contrast and the equinoctial or, better, monthly¹⁹ insolation values have to be considered in the modelling of the glacial-interglacial climate fluctuations in addition to or instead of the usual astronomical-caloric seasons frequently used.

Analysing the remaining terms in the trigonometrical expansion of $e \sin \tilde{\omega}$ and e , it seems that the next periods that could be found in geo-ecological records would be, for $e \sin \tilde{\omega}$ around 15,000 and 50,000 yr, for e around 21,000, 31,000 and 54,000 yr and, as far as a combined effect of both precession and obliquity is concerned,

59,000 and 64,000 yr. Discovery of these peaks would remove any remaining doubts about the validity of the astronomical theory of climatic change.

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A. L. BERGER

*Institut d'Astronomie et de Géophysique,
Université Catholique de Louvain,
Chemin du Cyclotron 2,
1348 Louvain-la-Neuve, Belgium*

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LaNi_{5-x}Al_x is a versatile alloy system for metal hydride applications

METAL alloys which reversibly absorb and desorb hydrogen are being used or considered for a variety of energy applications^{1,2}. Alloys of general composition AB₅ are prime candidates because of their good hydrogen absorption/desorption kinetics and large hydrogen storage capacity. The LaNi₅-H₂ system, in particular, has been extensively investigated. In many applications, however, materials are required whose plateau pressures are different from those of LaNi₅H_{6.7}. The desorption pressure of LaNi₅H_{6.7} may be modified by substitutions either of La or Ni with other elements. Previous work³ has shown that 20% substitutions of Ni by a variety of transition metals can lower plateau pressures by a factor of ~ 4 . We report here that Al can substitute for Ni in LaNi₅ with dramatic results in lowering decomposition pressures without impairing the kinetics or the hydrogen carrying capacities. The important new result is that Al substitutions allow a wide range of decomposition pressure to be spanned in continuous fashion. In the range 0-20% Al, the plateau pressures of the LaNi₅-LaNi₄Al hydride system are reduced by a factor of ~ 300 .

Alloys were prepared from metals of 99.9% purity by arc melting on a water-cooled copper hearth in an argon atmosphere. The alloys were homogenised at 800 °C. It was noted that diffraction patterns taken on as cast material were equally as sharp as those taken on homogenised alloys. X-ray diffraction patterns were taken with filtered FeK_α radiation. Computer programs were used to verify indexing of the X-ray patterns.

Alloy samples, weighed to ± 0.0001 g, were placed in an all 316 stainless steel reactor fitted with a 1 μ m porous stainless steel filter disk. The reactor was connected to an all 316 stainless steel high pressure manifold with connections to a 0-2,000 lb inch⁻² Heise pressure gauge (accuracy $\pm 0.1\%$), a vacuum pump, and a high pressure hydrogen gas cylinder. Hydrogen was Matheson's pre-purified grade (99.95% min). Hoke valves rated to 3,000 lb inch⁻² were used. The reactor was immersed in a water bath whose temperature up to 80 °C was maintained to ± 0.2 °C by use of a

Thermistemp temperature controller. For higher temperatures a nichrome wound cylindrical furnace was employed. Equilibration was considered to have been achieved when the pressure remained constant for a period of 10–15 min. Equilibration times were generally from 30 min to 2 h. Samples were 'activated' by evacuating the reactor containing the alloy and exposing the sample to high pressure hydrogen (300–800 lb inch⁻²) for 1–2 h. Desorption measurements were performed by removing measured amounts of hydrogen and re-equilibrating the system.

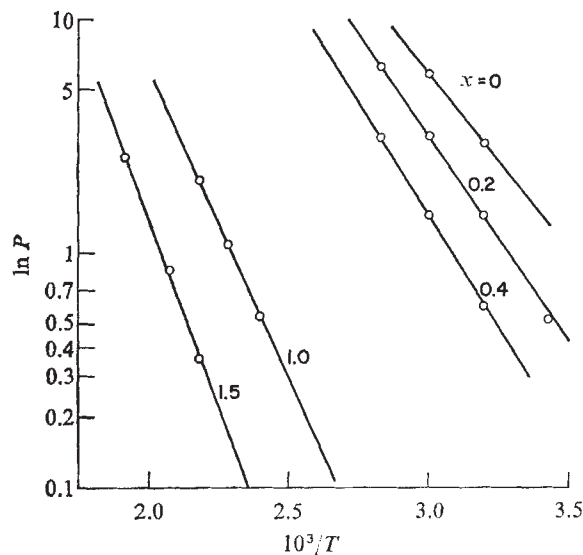


Fig. 1 Plot of $\ln P$ against $1/T$ for $\text{LaNi}_{5-x}\text{Al}_x$ hydrides.

Pressure–composition data have been obtained for alloys of the following composition: $\text{LaNi}_{4.8}\text{Al}_{0.2}$, $\text{LaNi}_{4.6}\text{Al}_{0.4}$, LaNi_4Al , and $\text{LaNi}_{3.5}\text{Al}_{1.5}$. Preliminary data at several temperatures allow us to plot $\ln P$ against $1/T$ for the hydrides of these four compounds. From the slope and intercept of the van't Hoff plots (Fig. 1), ΔH and ΔS values for the reaction



are estimated and given in Table 1.

The increasing stability of the hydrides can also be illustrated by isobaric data as in the last column of Table 1.

X-ray diffraction measurements have been made on a number of $\text{LaNi}_{5-x}\text{Al}_x$ alloys. These results, showing the a_0 and c_0 lattice parameters and the crystal cell volumes as a function of the Al concentration, are illustrated in Fig. 2. The substitution of one Al atom into the 5 c and g sites results in a sharp increase in c_0 , and a smaller increase in a_0 . Intensity calculations show that Al tends to enter the 3 g sites in preference to the 2 c sites. This finding is consistent with an earlier investigation of the $\text{YCo}_5\text{--YNi}_5$ system⁴. Further substitution of Al causes c_0 to level off but a_0 increases at a faster rate. The initial increase in c_0 must result from a lengthening of the bond between layers, while the subsequent levelling off in c_0 results from the beginning of a trend for the large La atom to collapse into the hexagonal ring of $\text{Ni}(\text{Al})$ atoms above and below it.

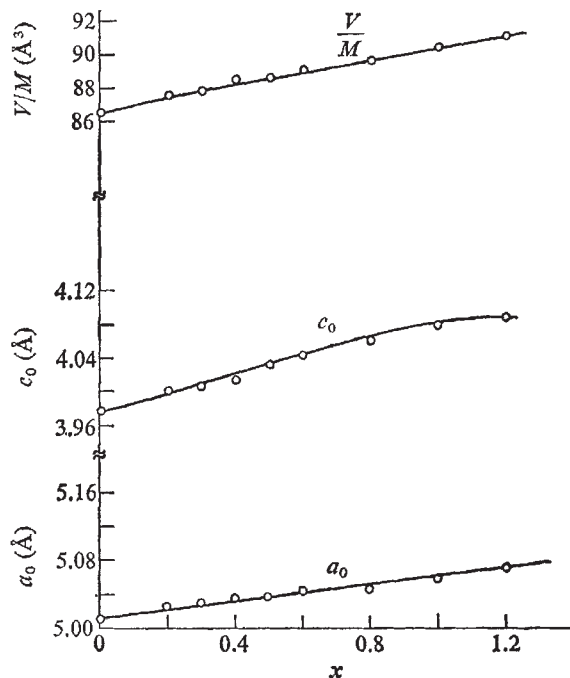


Fig. 2 Plot of a_0 , c_0 , and V/M against x in $\text{LaNi}_{5-x}\text{Al}_x$ alloys.

Several workers^{5,6,7} have pointed to the importance of crystal structure and geometrical considerations with regards to the stability of metal alloy–hydrogen systems. In Fig. 3, a direct linear correlation between $\ln P_{\text{plateau}}$ and the alloy cell volume is shown for the compounds: LaCo_5 , CeCo_5 , PrCo_5 , NdCo_5 , SmCo_5 , GdCo_5 , YCo_5 , LaNi_5 , PrNi_5 , SmNi_5 , GdNi_5 , LaCo_4Ni , LaCo_3Ni_2 , LaCo_2Ni_3 , NdNi_5 , $\text{LaNi}_{4.8}\text{Al}_{0.2}$, $\text{LaNi}_{4.6}\text{Al}_{0.4}$, and LaNi_4Al . The observed trend of lower plateau pressures with increasing Al content is in agreement with the cell volume correlation observed for other AB_5 compounds.

Fig. 3 Plot of $\ln P$ against alloy cell volume for AB_5 type hydrides, with ref. nos. See ref. 8 for LaCo_5 , CeCo_5 , PrCo_5 , NdCo_5 , SmCo_5 , GdCo_5 ; ref. 9 for YCo_5 ; ref. 10 for LaNi_5 ; ref. 11 for PrNi_5 , SmNi_5 , GdNi_5 ; ref. 12 for LaCo_4Ni , LaCo_3Ni_2 ; LaCo_2Ni_3 ; ref. 13 NdNi_5 ; the present work (ref. 14 on Fig.) for $\text{LaNi}_{4.8}\text{Al}_{0.2}$, $\text{LaNi}_{4.6}\text{Al}_{0.4}$, LaNi_4Al .

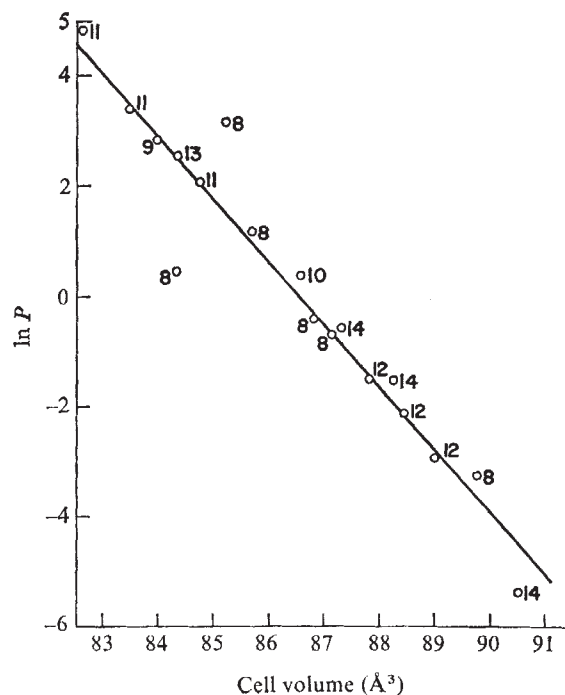


Table 1 Data from $\text{LaNi}_{5-x}\text{Al}_x + n\text{H}_2 = \text{LaNi}_{5-x}\text{Al}_x\text{H}_{2n}$ reaction

Compound	ΔH (kcal mol ⁻¹ H ₂)	ΔS (cal mol ⁻¹ H ₂ °C)	Temperature (°C) for $P = 2.0$ atm
LaNi_5	7.2 ± 0.1	26.1 ± 0.4	~25
$\text{LaNi}_{4.8}\text{Al}_{0.2}$	8.3 ± 0.1	27.3 ± 0.4	~50
$\text{LaNi}_{4.6}\text{Al}_{0.4}$	9.1 ± 0.2	28.1 ± 0.7	~70
LaNi_4Al	12.7 ± 0.3	29.2 ± 0.7	~180
$\text{LaNi}_{3.5}\text{Al}_{1.5}$	14.5 ± 0.6	29.6 ± 1.4	~240

A new ternary alloy system has been developed involving Al substitution of Ni in AB_5 compounds which permits control of hydrogen decomposition pressures over wide pressure and temperature ranges, while preserving the excellent hydrogen absorption-desorption kinetics and large hydrogen carrying capacity of $LaNi_5$.

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MARSHALL H. MENDELSON
DIETER M. GRUEN

Argonne National Laboratory,
Chemistry Division,
9700 South Cass Avenue,
Argonne, Illinois 60439

AUSTIN E. DWIGHT

Department of Physics,
Northern Illinois University,
DeKalb, Illinois 60115

Received 27 May; accepted 27 June 1977.

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Ni-Sn interaction in temper embrittled steel detected by Mössbauer spectroscopy

PREREQUISITES for reversible temper embrittlement of steel¹ are the presence of a trace impurity² (Sb, Sn, As or P) and a major alloying element^{3,4} (Ni, Cr or Mn). Auger electron spectroscopy (AES) demonstrated that in the embrittled state these elements are segregated to within the first few monolayers of the high angle boundaries^{5,6}. The segregation is reversible and can be removed by heating at temperatures above the embrittlement range, whilst still within the α field (that is $> 600^\circ\text{C}$). It has been postulated⁷ that the segregation behaviour can be explained using equilibrium thermodynamics. It is proposed that the trace impurities interact more strongly with the major alloying elements than the Fe matrix and hence the chemical potential of the system is reduced by segregation to the high angle boundaries with the formation of bi-dimensional complexes (such as Ni_3Sn_2 , Mn_3P and Cr_3P). No experimental evidence is available for the existence of such complexes. AES gives no information regarding the chemical bonding of the segregants but it has been shown⁸, using X-ray photoelectron spectroscopy (XPS), that the segregated Mn on embrittled fracture surfaces of a 2% Mn steel is in a different chemical state to that on unembrittled surfaces. We report here preliminary results from a specially prepared 3% Ni steel, doped with ^{110}Sn , which were obtained using Mössbauer spectroscopy to monitor changes in the nearest-neighbour atoms of the Sn atoms as a function of heat treatment in the temper embrittlement temperature range.

Argon arc melting was used to prepare the high purity alloy of composition (wt%) Ni (3.4); ^{110}Sn (0.19); C (0.10); Fe (balance). The cast alloy was rolled to a thickness of 100 μm , an optimum thickness for the Mössbauer measurements, and austenitised for 1 h at 1,120 $^\circ\text{C}$ followed by a water quench. Heat treatments were chosen to produce

Table 1 Heat treatment schedule

Condition	1120 $^\circ\text{C}$	650 $^\circ\text{C}$	500 $^\circ\text{C}$	650 $^\circ\text{C}$
As quenched	1 h, water quench			
Unembrittled	1 h, water quench	1 h		
Embripped	1 h, water quench	1 h	1,000 h	
De-embrittled	1 h, water quench	1 h	1,000 h	1 h

conditions representative of: the martensitic water quenched material, the unembrittled state, the embrittled state and the de-embrittled state, as detailed in Table 1. Mössbauer spectra were recorded in transmission at 77 K using a CaSnO_3 source and a constant acceleration spectrometer.

The Mössbauer spectra in Fig. 1 were found to consist mainly four components tentatively identified with Sn atoms having 0, 1, 2, or 3 Ni nearest neighbours. As the field in pure Fe is -8.5T and in Ni is $+2\text{T}$, the magnitude of the field is expected to decrease with increasing number of Ni nearest neighbours. At least-squares computer programme adjusted the intensities and magnitude of the fields of the four components to fit the spectra, and the percentages of the components are shown in Table 2. The errors are estimated to be $\pm 5\%$. With increasing number of Ni neighbours a progressive positive isomer shift was observed, indicating an increasing s electron density at the Sn nuclei. The total shift of 3 Ni neighbours was 0.22 mm s^{-1} .

For the as quenched material, the analysis showed a slightly greater percentage of Sn atoms having Ni nearest

Fig. 1 Mössbauer spectra obtained for a, the as quenched martensitic material; b, the unembrittled state and c, the embrittled state. On de-embritting, the spectra reverted to that of the unembrittled state.

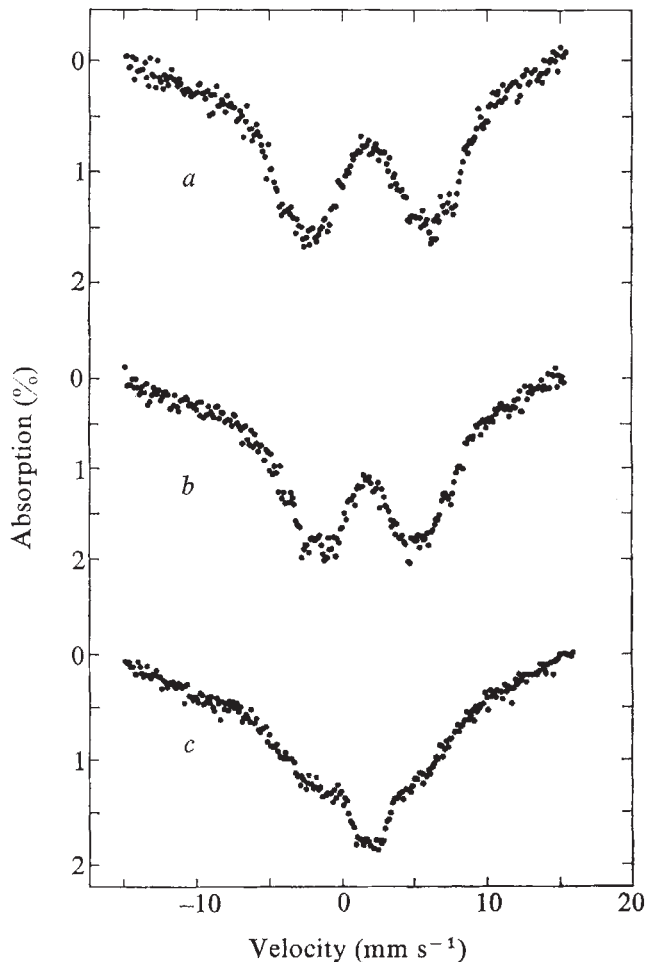


Table 2 Percentage of Sn atoms

No. of Ni nearest neighbours	0	1	2	3
Calculated value for random distribution (bcc)	76	21	3	0
As quenched	71	24	5	0
Unembrittled	62	32	10	6
Embrittled	33	23	22	22
De-embrittled	53	26	13	8
Field (T)	-8.5	-4.9	-2.1	-0.7

neighbours than that predicted from a random solid solution, indicating the presence of a Ni-Sn interaction within the γ field. Tempering at 650 °C (the unembrittled state) produced a further increase in the percentage of Sn atoms having Ni neighbours. On tempering at 500 °C, a dramatic change in the spectrum was noted and the analysis showed this is due to an increased number of Sn atoms having two or three Ni nearest neighbours. On re-heating at 650 °C (the de-embrittled state) these components were removed from the spectrum which reverted to that of the unembrittled state. The possibility of compound formation during tempering at 500 °C was examined by a comparison with the Mössbauer spectra produced by Ni_3Sn_2 or Ni_3Sn_4 . Within the accuracy of the experiments, the distinction between compound formation and Sn having three Ni nearest neighbours could not be resolved but is the subject of further investigation.

Examination of thin foils of the specimens in the embrittled condition using transmission electron microscopy failed to identify any compound formation either within the grains or at the grain boundaries. However, since AES⁹ has demonstrated that Ni and Sn are segregated at the boundaries of temper embrittled steels, it is reasonable to conclude that the modification of the spectra on ageing at 500 °C is due primarily to a grain boundary centred phenomenon associated with either the formation of a bi-dimensional phase of the type Ni_3Sn_2 (or Ni_3Sn_4) or due to an increased Ni-Sn coupling. The removal of this 'phase' on re-heating at 650 °C, above the embrittling temperature range, indicates that it has a critical role in the mechanism of temper embrittlement.

This work has demonstrated the existence of a reversible Ni-Sn interaction when subjected to a classical embrittling-de-embrittling treatment. This interaction is considered to be primarily centred at the grain boundaries and is related to either the increased number of Ni nearest neighbours of the Sn atoms or due to a bi-dimensional compound formation of Ni_3Sn_2 or Ni_3Sn_4 . Our work has shown that Mössbauer spectroscopy is a powerful tool for monitoring the environment of the Sn impurity atoms in temper embrittled steels as a function of heat treatment, and a similar evaluation may also be possible for Sb.

B. C. EDWARDS
B. L. EYRE

T. E. CRANSHAW

Metallurgy Division,

Nuclear Physics Division

Atomic Energy Research Establishment,

Harwell,

Didcot, Oxfordshire, UK

Received 13 June; accepted 14 July 1977.

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Water content of Russian tektites

THE first occurrence of tektites in Russia was reported recently by Florensky¹⁻³, who named them irgizites, and concluded that they were produced by fusion of terrestrial rocks at the Zhamanshin impact structure where they are found. The Zhamanshin impact structure is approximately 10 km in diameter and is 200 km north of the Aral Sea (~49°N, ~59°E). The irgizites have the splash forms that are commonly found among other tektites and impactites, for example, indochinites and Wabar glass. Although Florensky clearly describes the field occurrence, shapes, general petrography, major element chemistry and origin of the irgizites, the reported bulk chemical analysis of the major elements includes neither ferrous/ferric iron nor water³. Thus, data for two of the most widely accepted chemical criteria for the identification of tektites have not previously been available for irgizites. This note reports investigations into their water content.

Seven irgizites were obtained, one of which was available for destructive analysis. A thin plate was cut from this specimen, polished, and the water content of the glass was determined using the technique of Gilchrist *et al.*⁴ from the infrared absorption spectrum (Fig. 1). A moldavite and a terrestrial obsidian sample were run under the same conditions for comparison. The actual water determination for the irgizite was made on a slice 2.1 mm thick, and the value obtained was 0.051 weight per cent water. This figure is greater than the water content of the presently recognised tektites, which average approximately 0.01 wt % (ref. 4).

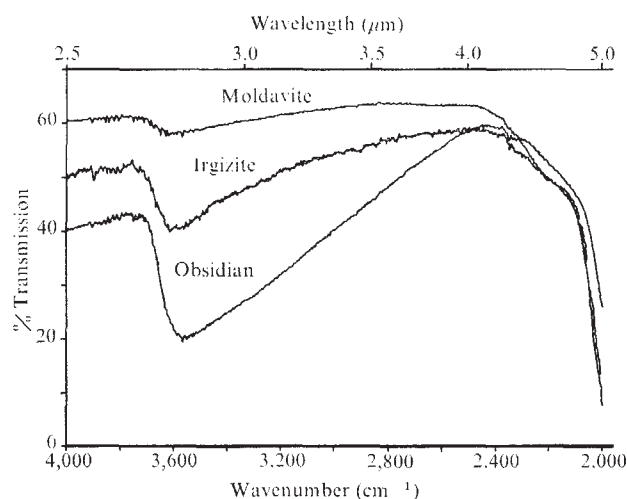


Fig. 1 Infrared absorption spectra of a moldavite, irgizite and an obsidian. Spectra were obtained from doubly polished sections 0.290 mm thick (moldavite), 0.275 mm (irgizite) and 0.180 mm (obsidian). The respective water contents are: moldavite, 0.0092; irgizite, 0.051; obsidian, 0.156 wt %. The water content of the irgizite is greater than that of true tektites.

In thin section, the body glass of the irgizite is a light olive brown colour, similar to many bediasites, and probably indicates a high ferrous/ferric iron ratio, but sufficient material was not available for a wet chemical determination. Partial electron microprobe analyses of the irgizite glass show a range of composition that is commonly found within a single tektite, and confirm the presence of lechatelierite inclusions³. The body glass of the irgizite is very clean with schlieren and slightly different colour and refractive indices; it also contains a number of spherical to elliptical bubbles, congruent in number and size with the bubbles in many tektites. There are no microlites nor any other traces of devitrification or

hydration. A few tiny and rare mineral grain inclusions are present, but they are too small to identify.

The piece of irgizite glass we examined seems to be a typical tektite glass in all respects except for the greater water content and possibly the, as yet undetermined, ferrous/ferric iron ratio. Whether or not the irgizites should be classified as tektites is a moot question. Perhaps they should be termed wet tektites, but regardless of their name, they are extremely similar to, and have originated by, the same basic process as true tektites.

Although the fundamental question of the place and mode of origin of tektites has been resolved for some years³⁻⁹, the irgizites are an important link in the chemistry and petrography of terrestrial impactites from craters of small to intermediate size.

The authors are indebted to Walter Zeitschel of Hanau for providing the specimens of irgizites used in this work.

ELBERT A. KING

Department of Geology,
University of Houston
and Mineralogical Institute,
University of Tübingen,

JORG ARNDT

Mineralogical Institute,
University of Tübingen,
74 Tübingen, Wilhelmstrasse 56, FRG

Received 17 June; accepted 30 June 1977.

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Source models to account for Lake Mungo palaeomagnetic excursion and their implications

INDICATIONS of large and rapid excursions in the direction of the ancient geomagnetic field continue to create considerable excitement and debate in palaeomagnetic circles. Barbetti and McElhinny¹ have published detailed evidence of a remarkable excursion at Lake Mungo, Australia dated at about 30,000 yr ago, on which they had briefly reported previously². I consider here various simple sources, all in the Earth's outer core, that could account for that excursion—an eccentric radial dipole or current loop, an eccentric horizontal dipole, and a pair of eccentric radial current loops of opposite sign—and discuss the merits of each in light of the modern geomagnetic field and with regard to the magnitude and spatial extent of the anomalous field produced.

Because most of the excursions proposed so far have been found in sediments and have not been convincingly documented over a sizeable portion of the globe, several workers have suggested that

some or most of them may reflect sedimentological rather than geomagnetic phenomena³⁻⁵. The record of the Lake Mungo excursion, however, is contained in sedimentary material that was baked in prehistoric aboriginal fireplaces. Thus, sedimentological phenomena cannot be invoked to explain the anomalous directions because the natural remanent magnetisation is not detrital or diagenetic in origin but rather is thermoremanent. This type of magnetisation is not only the most reliable recorder of ancient field direction but is also the best suited for making estimates of ancient field intensity.

The Lake Mungo excursion is especially intriguing because of the very high field intensity that Barbetti and McElhinny¹ have found accompanying the large swing in field direction. The intensity estimates are as high as 1.83 Oe, about twice as strong as anywhere on the surface of the Earth today and three times what would be produced at that latitude by the maximum geomagnetic dipole moment previously reported for the past 8,500 yr (ref. 6). While the field was abnormally intense its direction was shallowly inclined first roughly west and then roughly east, very far from the normal field direction of an approximately axial and centred dipole.

The explanation of this phenomenon with the least astonishing consequences is that some of the palaeointensity measurements are erroneously high. It is well known that ancient field intensities are far more difficult to determine than directions because of the many possibilities for systematic error⁷. Nonetheless, we cannot easily dismiss these extraordinarily high palaeointensities. Barbetti and McElhinny¹ carefully discuss the probable effects of a great number of possible systematic errors and conclude that their estimates of palaeointensity should be regarded as minimum values. Moreover, the high values were found at three sites in samples of two different sorts of baked sediment, whereas at several other sites these same materials yield both normal and unusually low estimates of palaeointensity. Thus, it is most useful at this point to accept the measurements of direction and intensity as at least approximately correct and to examine other explanations and their consequences.

The failure so far to find convincing evidence for any single excursion at widely separated points on the globe has led to the suggestion that excursions are manifestations of the non-dipole field^{8,9}. Likewise, Barbetti and McElhinny¹ propose that the most likely cause of the Lake Mungo excursion is the occurrence of a very large intensity non-dipole feature which may be modelled approximately by an eccentric, radially-oriented dipole in the core near the core-mantle boundary beneath Lake Mungo. They point out that such a radial dipole at 0.5 R (offset from the centre of the Earth by 0.5 of the Earth's radius) would need a moment of only one-eighth that of the main geocentric dipole to produce at the point on the surface nearest to it a field greater than or equal to that of the main dipole, whereas on the far side of the Earth it would produce a field only 1/27 as large.

Even higher field intensities, however, are required elsewhere on the surface of the Earth when one attempts to apply the radial dipole model in a quantitative manner to the Lake Mungo excursion (Fig. 1a). To do this, I have assumed that the dipole field at the time was axial and had an intensity 0.3 Oe at the site. This is the most reasonable assumption in accordance with the data¹, but

Table 1 Solutions for radial dipoles corresponding to Lake Mungo excursion

Site	Non-dipole field			Nearer solution				Farther solution			
	F_N	F_E	F_D	λ	ϕ	M/M_0	θ	λ	ϕ	M/M_0	θ
F7*	-0.68	-0.76	0.27	-52	81	-1.4	48	13	186	2.3	62
F12	-0.44	-1.81	0.14	-29	80	-3.1	53	-8	199	3.6	57
F8	-0.06	0.57	0.14	-26	198	-0.8	48	-11	80	1.3	62
F9*	-0.37	1.18	0.25	-34	203	-1.8	49	-3	87	2.7	60

Sites are those of Barbetti and McElhinny². F_N , F_E , F_D are north, east, downward non-dipole field components in Oe (10^{-4} T). λ , ϕ , and M/M_0 are north latitude, east longitude, moment divided by present geocentric dipole moment of radial dipole. θ is great-circle angle in degrees between radial dipole and site.

*Palaeointensity is average of values derived from ovenstone and baked sediment².

other plausible choices such as the present inclined dipole field of approximately 0.6 Oe at Lake Mungo do not seriously change the results. The differences between the palaeomagnetically determined total field vectors and the presumed dipole field vector are the ancient non-dipole field vectors (Table 1), for which one seeks to find the appropriate radial dipole sources.

Two possible solutions always exist for a radial dipole at any particular depth that can produce a given field at a site on the surface. These can be found using standard formulas¹⁰ and an iterative technique. The dipole nearer to the site naturally has a smaller moment than the one further away that produces the same field. Thus, it is the nearer dipole that is the more likely to correspond to the real physical situation if the non-dipole field at the site is in fact produced by only one such source. The depth of the radial dipole that is best used depends on the width of the anomaly to be modelled at the surface. Assuming non-dipole features in the past to have been similar to those of today, offsets of 0.35–0.45 R are most appropriate. These correspond to half-widths of 44–56° great circle arc for the vertical component of the anomaly.

The solutions for the positions and strengths of radial dipoles with offset 0.40 R that would produce the non-dipole field vectors for the Lake Mungo excursion are given in Table 1. The nearer solutions are inward-pointing dipoles at southerly latitudes of 26° or higher and at longitudes around 80°E or 200°E for the sites with westerly or easterly palaeomagnetic declinations, respectively. The farther solutions are only about 11° more distant on the average than the nearer solutions, and are outward-pointing dipoles lying within 13° of the equator at longitudes around 190°E or 85°E. The solutions are listed in order of presumed decreasing age and span a period of 2,000–3,000 yr (ref. 2).

The moments of even the nearer radial dipoles are enormous, ranging from 0.8–3.1 times the present geocentric dipole moment, and the alternate farther dipoles are up to 60% stronger than the nearer ones. These hypothetical dipoles would cause extremely large intensities at the points on the surface nearest to them of 2.3–8.8 Oe. Even at the antipodal points the intensities are about 0.2–0.7 Oe, certainly not negligible compared with usual field intensities.

Shifting the radial dipole toward or away from the centre of the Earth does little to diminish the excessively high field intensities. A radial dipole just below the core–mantle boundary (0.54 R) or just above the inner core (0.20 R) that produces the largest non-dipole field vector inferred for Lake Mungo (that at site F12, Table 1) would produce field intensities over the whole Earth ranging from 10.6–0.3 Oe or 6.1–1.8 Oe, respectively. Likewise, replacing the simple point dipole by a distributed source such as a circular loop

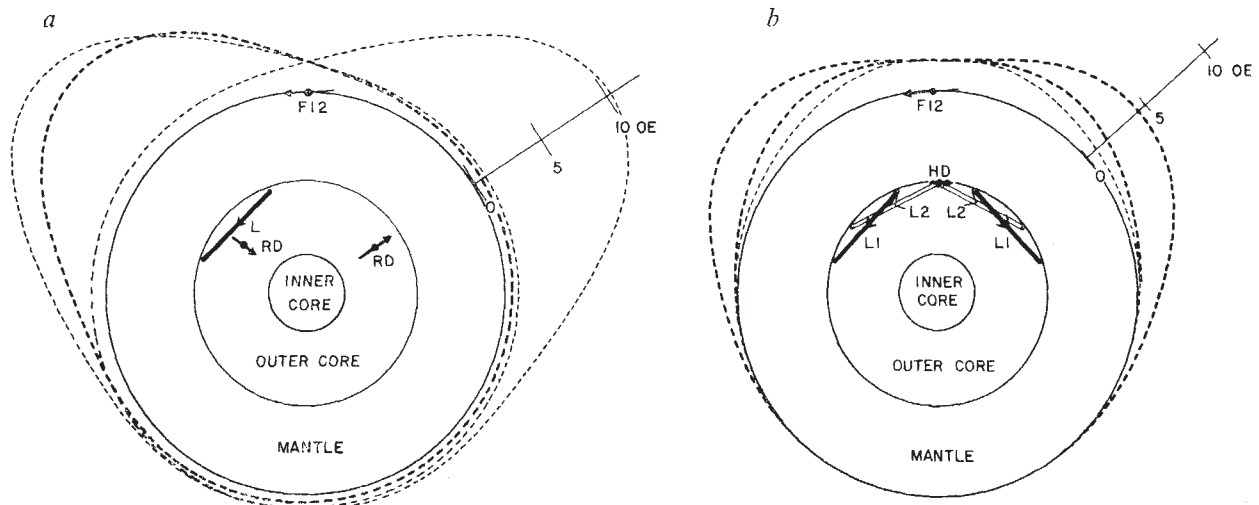
of current at the core–mantle boundary is only a minor improvement. Such a current loop with radius 44% of the radius of the core, which would cause an anomaly at the surface of about the same width as a radial dipole at 0.40 R, would bring about intensities over the world ranging from 7.2–0.4 Oe, while producing the largest non-dipole field vector inferred for Lake Mungo. Variations of the total field intensity for various of these models are illustrated in Fig. 1a.

The only way for such a large, almost horizontal non-dipole field as apparently occurred at Lake Mungo to exist without much larger fields existing simultaneously elsewhere on the Earth is to relax the assumption that the cause was a simple, radial source. When two adjacent radial sources have opposite signs, their horizontal components add between them and the area dominated by their combined fields is much less than for a single source. For example, consider the current loop described above and a slightly stronger current loop of opposite sign, 96° away from the former on the other side of Lake Mungo, that would also produce the same field at site F12. If reduced to half their strengths, the two loops together would likewise cause the required non-dipole field at site F12, but over the rest of the Earth they would produce fields that varied from only 4.5 to almost 0 Oe (Fig. 1b). If the current loops were allowed to overlap slightly, the most efficient configuration would be two current loops of equal strength and opposite sign spaced 54° apart. Such a pair would cause fields that varied from only 2.3 Oe to exactly 0 Oe over the globe (Fig. 1b), while producing the required 1.87 Oe at site F12. The spacing of 54° lies at the short end of the observed range of about 50–100° for the spacing of adjacent features of opposite sign of the modern non-dipole field¹¹.

The other assumption that might be relaxed is that the source is radial. A horizontal dipole at the core–mantle boundary would only require a strength of about 20% or less of the present main geocentric moment to account for the non-dipole fields inferred at Lake Mungo. This dipole would cause field intensities over the globe varying from about 2.0 Oe to less than 0.05 Oe (Fig. 1b). Combinations of two horizontal dipoles could reduce still further the maximum field intensities and the area of the globe significantly affected.

What, then, is the most likely source for the very high intensity Lake Mungo excursion? A horizontal eccentric source or current loop is the most efficient in terms of causing the least disturbance of the field elsewhere around the world, but there is little basis for expecting one to exist since the modern non-dipole field is dominated by a few large features that are most simply modelled by radial sources^{10,11}. Single radial sources, however, would produce

Fig. 1 Magnetic field intensity at Earth's surface due to various sources in outer core. Dashed lines indicate total field, zero if coincident with surface of Earth. Each source produces the palaeomagnetic non-dipole field at Lake Mungo site F12 (Table 1). *a*, Single radial sources: radial dipole at 0.40 R (RD), light dashed lines; current loop (L), heavy dashed line. *b*, Paired radial sources or horizontal source: current loops of opposite sign (L1 or L2), heavy dashed lines; horizontal dipole (HD), light dashed line.



extremely high fields over very large areas and significantly high anomalous fields over the entire globe. Thus, they are improbable unless simultaneous worldwide effects are discovered. The most appealing explanation seems to be a combination of two radial eccentric sources of opposite sign. In their most efficient configuration such a pair would cause a maximum field of about 2.3 Oe, while producing the required non-dipole field at Lake Mungo (site F12), and significant effects would occur as far away as south-eastern Asia and India, southern Africa, and much of Antarctica and the South Pacific Basin.

To produce all four non-dipole fields listed in Table 1, such a pair of sources would have to change signs and move about 15° N and S, in a similar fashion to the oscillating radial dipoles of fixed average position recently postulated by Creer¹² in explanation of geomagnetic secular variation. Although it could well be coincidental, it is interesting that two of the oscillating dipoles proposed by Creer lie within 10° and 25° of the average positions of the optimally-spaced pairs of current loops calculated to produce the field directions of the Lake Mungo excursion.

Obviously, there is a critical need for additional records of the Lake Mungo excursion in other places around the world. Only these will provide firm answers to fundamental questions such as whether the excursion is an expression of the dipole or non-dipole field and whether the very high field intensities inferred from the experimental data are in fact real. If the non-dipole field is responsible, such records are also necessary to determine the shape, number and configuration of the sources. At present, one can only conclude that if the very high field intensities are approximately correct and if the excursion was caused by non-dipole features similar in configuration to the modern ones, then unusually strong non-dipole fields must have occurred simultaneously over a significant portion of the rest of the world.

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R. S. COE

Earth Sciences Board,
University of California,
Santa Cruz, California 95064

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Trace elements in zooplankton particulate products

It has become increasingly evident that biogenous processes in oceanic surface layers play an important role in removing trace elements from sea water and transporting them to the sediments^{1,2}. Plankton are strongly implicated in these processes and investigations of the chemistry of these organisms have demonstrated their ability to accumulate trace elements to relatively high levels^{3,4}. Zooplankton metabolic activity can be expected to enhance the biogeochemical cycling of trace elements through the release of particulate matter⁵ such as faecal pellets, moulted exoskeletons and eggs. Furthermore, theoretical models^{3,6} on the vertical flux of particulates have emphasised the sinking of large particles, similar to those produced by zooplankton, as a mechanism for rapid transport of particulate matter

and associated trace elements out of the mixed layer. Nevertheless, little, if any, data exist on the trace element composition of these biogenic particles, and Boyle *et al.*⁷, interpreting their Pacific Ocean Cd profiles in terms of probable Cd regeneration from sinking biogenic debris, have stressed the need for information on the trace element composition of planktonic particulate matter. This note reports the concentrations of 18 trace elements in freshly released faecal pellets, moults and eggs from a representative planktonic crustacean. The high levels of many trace elements found in these biogenic products, relative to concentrations in the plankton which produce them, clearly indicate the importance of these particulates in oceanic trace element biogeochemical cycles.

Many euphausiids (*Meganyctiphanes norvegica*) were collected during one night in the surface waters off Monaco by making several, short oblique tows with a midwater trawl. Approximately 1,000 individuals were placed in a faecal pellet collection system⁸ and several hundred milligrams (wet) of pure faecal pellets were retrieved after 15 h. The remaining euphausiids were split into two groups; one was maintained in the laboratory⁹ until a sufficient number of moults had been collected and the other was killed for whole body analysis. Eggs released by females were concentrated from the water on fine mesh nylon netting. Microplankton, on which *M. norvegica* were feeding, were collected at the same time for purposes of comparing their trace element contents with levels in the euphausiid faecal pellets. All samples were briefly rinsed with doubly distilled water to remove adhering sea salts and oven-dried at 60 °C. Separate aliquots of material intended for Hg analysis were freeze-dried in order to avoid Hg loss through volatilisation. Care was exercised to avoid metal contamination at all stages of collection and sample preparation. Microplankton samples were carefully screened for possible metal-particle contamination using the Martin and Knauer⁴ method. Samples along with appropriate blanks and standards were analysed by flameless or flame atomic absorption spectrophotometry¹⁰ and instrumental neutron activation¹¹.

Trace element concentrations in euphausiids, their particulate products and the food they eat are given in Table 1. Since all samples were derived from the same water mass, realistic comparisons can be made between trace element levels in the particulates and those in the organisms which produced them. For most elements, faecal pellets contained the highest trace element levels with lesser amounts in moults and eggs. Trace element concentrations in faecal pellets were significantly higher than levels in whole euphausiids (as much as 1,000 times) except for Sr and possibly Hg and Se. The natural diet of *M. norvegica*, an omnivore, is extremely varied¹² but consists primarily of phytoplankton, small zooplankton and detritus. Although it is difficult to define precisely the exact composition of their diet at any given moment, examination of euphausiid stomach contents and faecal pellets indicated that the samples were feeding on many of the species noted in the microplankton. Comparison of the trace element content in microplankton and euphausiid faecal pellets in Table 1 shows that, except in the case of Sr, euphausiids readily concentrate ingested trace elements in their faeces. Faecal pellet-microplankton concentration ratios ranged as high as 670 with the greatest degree of concentration noted for trace elements not biologically essential such as Ce, Sb, Cs, Eu and Sc. The high concentration ratio for Fe, a biologically essential element, is possibly a result of ingesting a particulate form of this metal which may not be easily assimilated. Boothe and Knauer¹³ have also reported a relatively high concentration of Fe in crab faeces relative to that in their food.

With the exception of Cu, Cs, Hg and Se, trace element concentrations in moults were also significantly higher than

Table 1 Trace element concentration ($\mu\text{g g}^{-1}$ dry) in a planktonic euphausiid, its particulate products and the microplankton it ingests

Sample	Wet-dry weight ratio	Ag	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Ce*	Cs*	Eu*	Hg*	Sb*	Sc*	Se*	Sr*
Faecal pellets	4.4	2.1	9.6	3.5	38	226	24,000	243	20	34	950	200	6.0	0.66	0.34	71	2.8	6.6	78
Moult	4.6	2.9	2.1	0.80	5.3	35	232	11.7	6.7	22	146	1.2	0.019	0.0077	0.17	0.80	0.030	1.9	350
Eggs†	10.0	0.96	0.58	0.80	7.9	17	330	11.5	4.3	8.9	318								
Whole euphausiid	4.7	0.71	0.74	0.18	0.85	48	64	4.2	0.66	1.1	62	0.21	0.062	0.0023	0.35	0.071	0.0090	4.4	117
Microplankton‡	10.7	0.67	2.1	0.87	4.9	39	570	17.9	8.1	11	483	0.30	0.080	0.013	0.05	0.22	0.13	2.7	520

*Determined by instrumental neutron activation analysis.

†Insufficient sample for neutron activation analysis.

‡Principally copepods, phytoplankton, chaetognaths and detritus retained on nylon netting of 76 μm aperture.

corresponding levels in whole individuals (Table 1). Bertine and Goldberg¹⁴ analysed several trace elements in shrimp and found that Zn and Ag were higher in dissected exoskeleton than in internal tissues. When small pelagic crustaceans moult, only the thin outer layer of the exoskeleton is shed. If surface adsorption predominates in the accumulation of many of the trace elements on to chitinous moults, as is the case for several radionuclides¹⁵, it is conceivable that moults will contain higher concentrations of these elements than the entire exoskeleton.

M. norvegica moults average 7.7% of the organism's dry weight; from this, rough estimates of the fraction of the animal's trace element content contained in the moult can

than moults in effecting element transport, since egg deposition is seasonal. Zooplankton faecal pellets, which are produced at a higher rate¹⁹ and decompose more slowly²⁰ than moults, clearly have the greatest potential for transporting trace elements to depth. Recent finding of large numbers of intact faecal pellets in deep waters^{21, 22} supports this contention. Trace element flux rates resulting from the release of moults and faeces by euphausiids can be calculated and are given in Table 3. From a comparison of the rates it is clear that, with the exception of Sr, faecal pellet flux is a major contributor to the biogenic vertical transport of the elements investigated. Thus, vertical transport through faecal pellets may be an important factor

Table 2 Fraction of euphausiid trace element body burden contained in the moult

	Ag	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Ce	Cs	Eu	Hg	Sb	Sc	Se	Sr
% in moult*	31	22	34	48	6	28	21	78	≈150	18	44	2	26	4	87	26	3	23

*Calculations based on p.p.m. dry weight values listed in Table 1.

be calculated (Table 2). Clearly, for many trace elements, a large fraction of the euphausiid's total body burden is associated with its outer surface. The reason for the anomalously high Pb percentage is not readily explainable and measurements made on another set of samples led to a similarly high percentage. The possibility of contamination can not be entirely ruled out; however, Pb is known to be highly concentrated on the epidermis of marine fish¹⁸, and

influencing the oceanic residence times of these elements. The degree to which faecal pellets and other biogenic debris transport their trace element load to depth will be a function of trace element regeneration time for these sinking materials. Considering the high trace element content of freshly released zooplankton particulate matter, some attempt to understand *in situ* trace element regeneration kinetics in these products is needed.

Table 3 Trace element flux rate contribution (μg per kg euphausiid per d) by euphausiids*

	Ag	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Ce	Cs	Eu	Hg	Sb	Sc	Se	Sr
Faecal pellets	80	360	130	1,400	8,600	910,000	9,200	760	1,300	36,000	7,600	230	25	13	2,700	110	250	3,000
Moult	26	19	7	48	320	2,100	110	60	200	1,300	11	0.2	0.07	1.5	7	0.3	17	3,200

*Based on typical *M. norvegica* faecal pellet and moult production rates¹⁹ of 0.038 and 0.009 g dry matter per g dry euphausiid per day, respectively. Flux due to egg deposition is not considered since egg production takes place for only a brief period during the year¹⁹.

it may be that the cast moult adsorbed additional Pb on its inner surface before it was removed from the water. Nevertheless, the implication is that most or all of the Pb associated with euphausiids is located on the surface of the exoskeleton.

The relatively high concentrations of so many trace elements in zooplankton particulates hold important implications for marine biogeochemical cycles. Pelagic crustaceans moult as often as every few days^{9, 17, 18}, in some cases throughout their entire lifespan. Consequently, sinking moults may either release trace elements to the deeper waters or sediments during decomposition or, if eaten, provide a rich source of trace elements to organisms that consume them. Released eggs, often also displaying a relatively high trace element content, will be much less important

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SCOTT W. FOWLER

International Laboratory of Marine Radioactivity,
Musée Océanographique, Monaco

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Weis-Fogh clap and fling mechanism in *Locusta*

WEIS-FOGH discovered a mechanism of lift generation¹ when he observed, from slow motion films, that the tiny parasitic wasp *Encarsia* clapped its wings together at the top of the stroke during hovering flight; the clap was followed by a 'flinging open' of the wings, as they rotated about a common axis where the hindwing trailing edges were in contact. He calculated that the insect generated greater lift than could be explained by steady-state aerodynamics. The importance of the clap and fling in generating this extra lift is now well established^{2–4}. We report here that slow motion film of natural flight has revealed this mechanism in locusts and that it occurs during forward flight to produce extra lift.

Swarms of *Locusta migratoria* were filmed with a high-speed camera in Australia and New Guinea. Film taken pointing directly towards an approaching and climbing swarm revealed that the hindwings of the locusts met at the top of the stroke in a similar fashion to that observed by Weis-Fogh in *Encarsia*. During the clap the tips of the hindwings meet, leaving a characteristic gap lower down towards

the wing bases. At the beginning of the downstroke, the leading edges of the hindwings part, but the more posterior vannal portions remain touching; as the hindwings move further down, the two wings eventually become completely separated from one another and they then progress as in a normal stroke. Significantly this clap and fling stroke is seen only in climbing locusts, which need greater lift than locusts in horizontal flight. Aspects of the stroke can be seen in Fig. 1.

The hindwing movement on the upstroke, before the clap, is noticeably faster than in a non-clapping stroke, and Fig. 2a shows this remarkably rapid upstroke which can be twice as fast as its forewing counterpart; hindwing tip speeds up to 10 m s^{-1} have been calculated from the film. Differences in phase between the fore and hindwings during the clap stroke seem to be essentially similar to those in the normal non-clap stroke (see Fig. 2b), and thus the hindwings move through a greater amplitude before the clap simply by moving faster than usual, reaching the top of their stroke with about the same phase lead over the forewings as in a non-clap stroke. Only the amplitude difference between fore- and hindwings changes markedly, and this results in a characteristic configuration with the hindwings pointing vertically and the forewings pointing horizontally (see Fig. 1).

On some of the high-speed film an occasional butterfly (unknown species) was seen flying across the field of view. Like the locusts, they also performed a wing clap at the top of the stroke⁴; in some cases the clap together was not immediately followed by separation as in the locusts, and instead the wings were held closed for a fraction of a second. The clap was observed both in approximately horizontal and vertical flight.

The clapping of the locust hindwings might be expected to make a noise, as is the case with pigeons taking off⁵. There are several reports of locust flight noises, especially during take-off⁶. Experiments performed in the field^{7,8} showed that the hindwings were responsible, and variously attributed the noise to the interaction of hindwings with back legs⁹, or to stretching of the vannal area of each

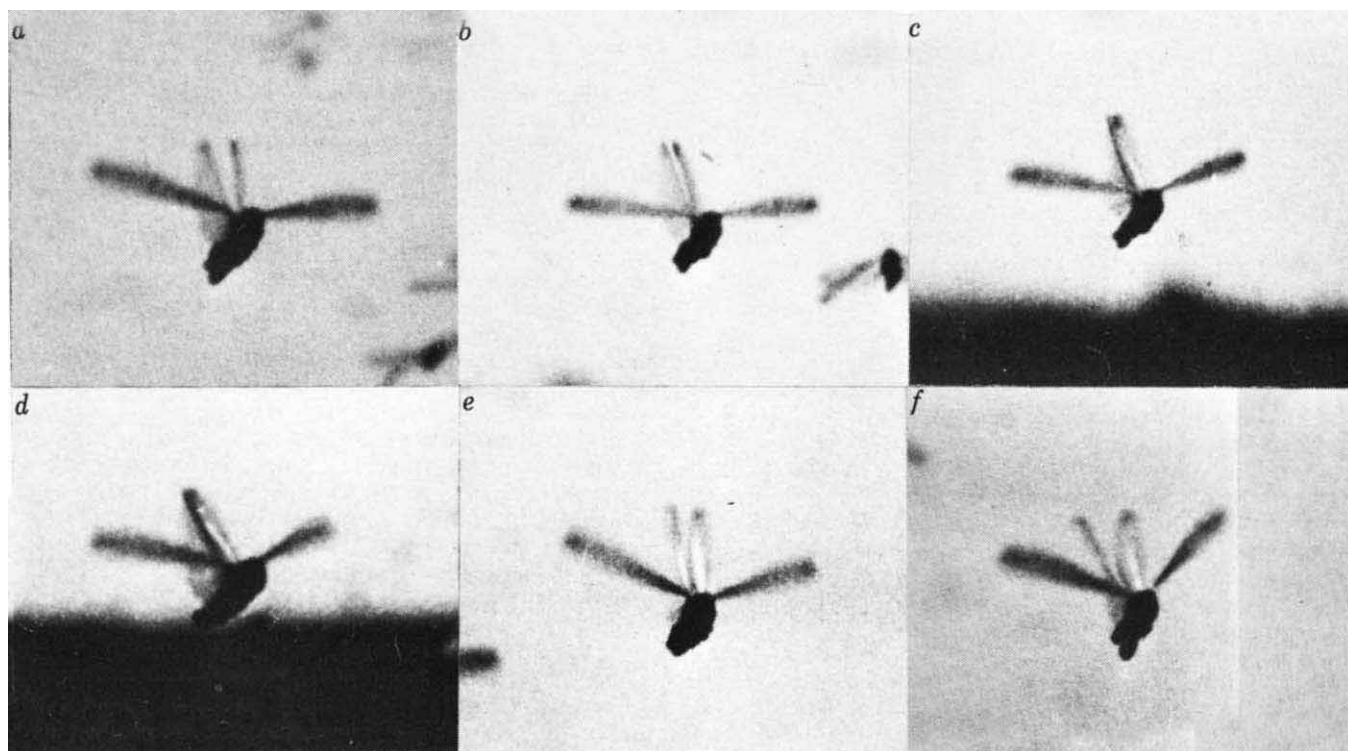


Fig. 1 Sequence of hindwing clap and fling in *Locusta*. Wings meet directly above the body and then at the start of the downstroke, the leading edges open, leaving more posterior portions of the wing still in contact. (Each frame is taken from a different locust.)

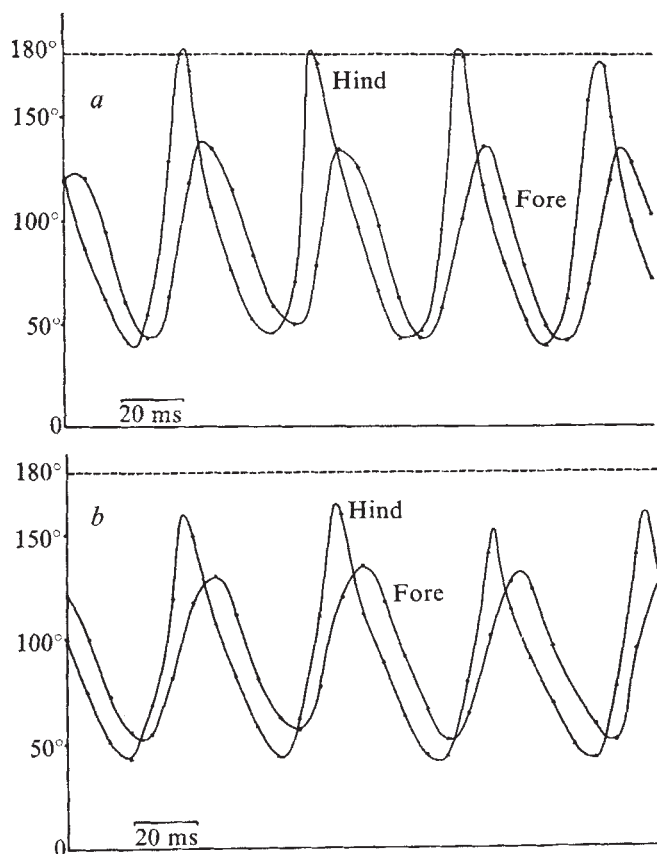


Fig. 2 *a*, Wingbeat cycles of right fore and hindwings of a locust performing clap. *b*, Wingbeat cycles of a different locust during non-clap strokes. 180° refers to wings in vertical clap position, and thus 90° is wings in horizontal position.

hindwing¹⁰. We suggest, however, that the noise is caused by the hindwings clapping together, this being likely to make a louder noise than the more proximal parts of the hindwings striking the legs. In the case of the locust *Dissosteira carolina*¹¹, which can apparently produce noise whilst hovering over a nearly fixed point, it performs a type of aerial dance and noise display thought to be of a sexual nature; we therefore suggest that the clap and fling mechanism may have a secondary sexual function in this case.

The significant aspect of these findings, both in locust and butterfly, is that the clap and fling mechanism seems to be used in forward flight as well as hovering, and thus must now be recognised as an aspect of primary importance in insect flight.

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R. J. COOTER
P. S. BAKER

Centre for Overseas Pest Research,
London W8, UK

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Post-metamorphic eye migration in *Rana* and *Xenopus*

CONTRADICTORY results have been obtained with *Xenopus laevis* and *Rana pipiens* in experiments directed at evaluating the possible role of visual experience in assuring proper binocular relations in the optic tectum. Gaze, Keating, and colleagues, working with *Xenopus*, reported that, in certain circumstances, experimental alteration of the contralateral visuotectal projection by eye rotation results in a modification of the ipsilateral projection so as to re-establish appropriate binocular relations^{1,2}. Jacobson and co-workers, however, working with *Rana*, reported no change in the ipsilateral projection after experimental rotation of the eye^{3–6}. Based on the hypothesis that the ability to respond to experimental perturbation of interocular relationships would correlate with the amount of variation in interocular relationships occurring normally, we predicted that *Rana* might exhibit substantially less post-metamorphic eye migration than *Xenopus*. We report here results which confirm this prediction.

In both *Xenopus*⁷ and *Rana*³, the ipsilateral visuotectal projection develops during metamorphosis, when the eyes migrate from lateral positions, with little or no overlap of their visual fields, to positions with significant binocular overlap. Because we were concerned with possible changes in interocular relationships occurring after the ipsilateral projection has developed, we concentrated our attention on relative eye position from the completion of metamorphosis onwards. Keating⁸ drew attention to the fact that eye migration continues for some period post-metamorphically in *Xenopus*. We here provide quantitative confirmation of a continual change in relative eye position in post-metamorphic *Xenopus*. In contrast, we have found that relative eye position is stable post-metamorphically in *Rana*. The photographs in Fig. 1 illustrate these basic conclusions. Figure 1*a* shows the relative eye position in a *Xenopus* froglet in the final stage of metamorphosis, as well as in an older juvenile and in an adult. The eyes continue to migrate dorsally, towards each other, after the end of metamorphosis. Similar photographs, however, for the *Rana* froglet and adult (Fig. 1*b*) show strikingly similar relative eye positions.

To quantitate these observations we devised a modification of the techniques used by Fite⁹ to determine the extent of visual fields with relation to body axes in frogs and toads. *R. pipiens* and *X. laevis* were reared in the laboratory from fertilised eggs and adults were obtained commercially. At known metamorphic stages or post-metamorphic ages individuals were either anaesthetised or paralysed and mounted at the centre of and facing towards an Aimark projection perimeter. An ophthalmoscope held in a moveable clamp was used to illuminate the eye and to visualise light reflected from within it. Once this was done the ophthalmoscope was moved peripherally in the visual field until the reflected light just disappeared. The ophthalmoscope was then kept in this position while a mirror was used to intercept the light beam and reflect it back through the ophthalmoscope onto the perimeter where its position was noted as an angle with respect to body axes. Repetitions of this procedure for both eyes with different directions of movement of the ophthalmoscope outlined the extent of the anterior half of the two visual fields. The whole process was then repeated with the animal facing away from the perimeter, giving the posterior half of the visual fields.

Results are shown in Fig. 2. In all cases, data from four or more animals have been included to indicate the range of variations which along any meridian or

parallel rarely exceeds 10° . A fair amount of this variation is attributable to uncertainties in the procedure itself, since repeated determinations in a single animal give comparable variations. It is clear from Fig. 2a and b that there is little or no difference in relative eye position between an immediately post-metamorphic and an adult *Rana*. For *Xenopus*, however, there is a significant amount of dorsal movement of the eyes subsequent to metamorphosis (Fig. 2c, d). The migration involves a shift of the visual axis of about 30° , is half completed at 7 wk, and still in progress at six months (Fig. 2e). There is apparently no substantial change in monocular visual field size or shape over this period.

The significance of the continuing eye migration in *Xenopus*, as pointed out by Keating⁸, is that, unless there is complicated reorganisation of the retinae, the region of one retina which is directed at the same part of visual space as a given region on the other is constantly changing. In these circumstances constant readjustment of the binocular interactions by the use of visual experience may be an essential developmental mechanism for maintaining the appropriate interactions between the two eyes. The results of experimental rotation of one eye may be interpreted as indicating the presence of such a mechanism operating during normal development. In *Rana*, however, as we have shown, the developmental problem of constant readjustment of binocular interactions is not present or, at least, is very much less. A mechanism for matching

Fig. 1 Photographs illustrating eye position at metamorphic climax and subsequently in a, *X. laevis* and b, *R. pipiens*. In each case, the smallest animal is one just at the completion of metamorphosis and the largest is 1 yr or more post-metamorphic. For *X. laevis*, an additional animal aged 4 wk post-metamorphosis is also shown.

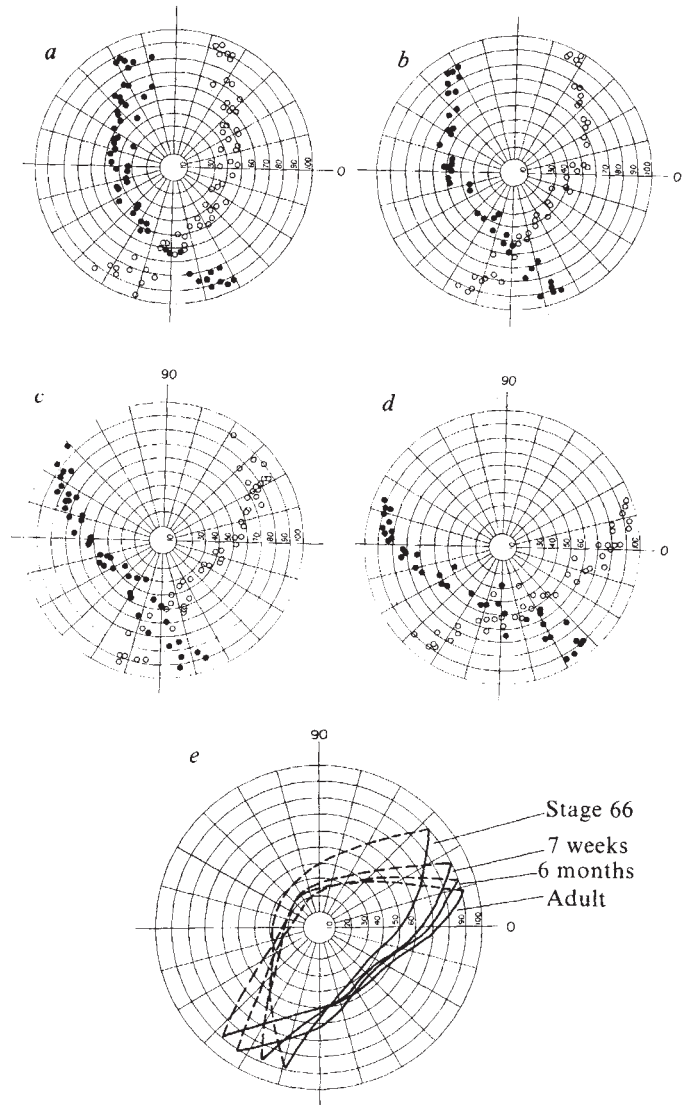
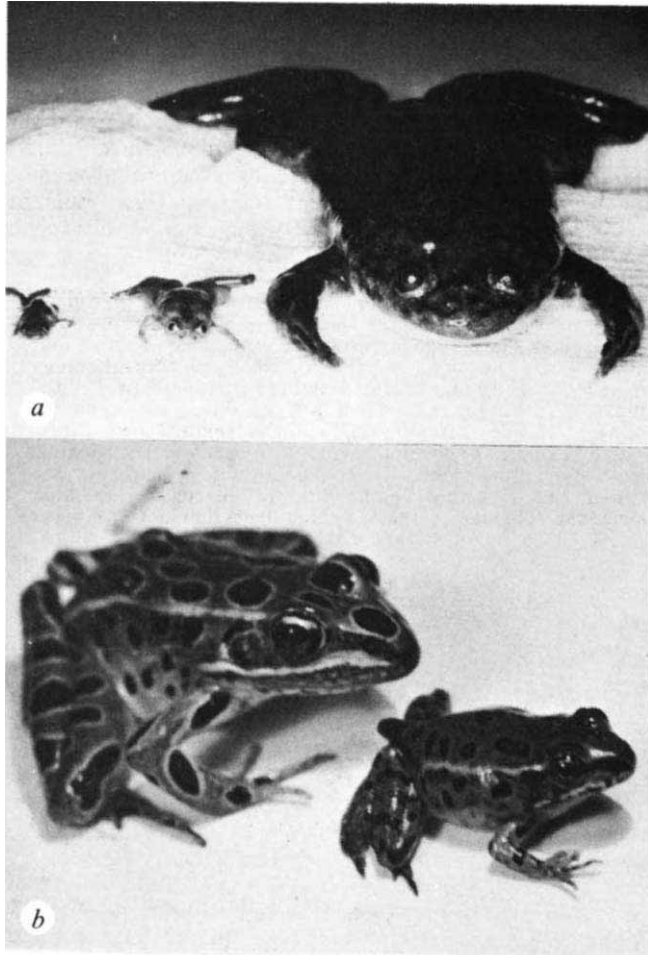


Fig. 2 Visual field boundaries for *R. pipiens* and *X. laevis*. Anterior visual field boundaries for the left (●) and right (○) eyes are shown for metamorphic climax *Rana* (a) and adult *Rana* (b) and for *Xenopus* (c, metamorphic climax; d, adult). Each map represents a hemisphere with the centre raised from the page. The animal should be pictured as sitting inside the hemisphere and facing the reader. The animal's eyes are in the plane of the page and centred on the intersection of planes corresponding to the vertical and horizontal meridia of the map. The symbols are actual border points for several different animals obtained as described in the text. Similar maps obtained for the posterior visual field boundaries are not shown. Data are from five animals in a, c and d and four in b. From maps like these, average visual field boundaries were estimated to illustrate the time course of eye migration in *Xenopus* (e). In (e), the visual field boundary of only the right eye of animals at the indicated ages is shown. The map, however, should this time be interpreted as a complete sphere with the posterior borders of the visual field (broken lines) being seen through the transparent anterior hemisphere.

the ipsilateral to the contralateral visuotectal projections may be correspondingly absent or significantly less effective and hence not lead to the same sequelae to experimental eye rotation as in *Xenopus*. Following this line of argument, our findings not only provide a possible explanation for the discrepant experimental results in *Rana* and *Xenopus* but also support the suggestion of Keating that visual experience is important in assuring proper binocular interaction in conditions of continuing eye migration⁸.

On the other hand, the differences between *Rana* and

Xenopus may be more quantitative than qualitative. In reviewing the mammalian literature, Grobstein and Chow^{10,11} put forward the proposition that binocular interaction may necessarily depend on visual experience because developmental variability makes genetic information inadequate to assure proper pairing of retinal regions. They also suggested that the influence of visual experience is probably constrained to some restricted range of operation by the genetically based developmental program (a suggestion earlier made by Shlaer¹²). It may well be that the range of operation in *Xenopus* is quite large, corresponding to the range of relative eye positions over which the system must operate, whereas in *Rana* it is quite small. Thus, the fairly large eye rotations which are necessary experimentally to be sure that a rotation has been accomplished lead to a compensation of the ipsilateral projections in *Xenopus* but not in *Rana*. It would be of interest to know whether the ipsilateral projection in *Rana* will compensate following very small eye rotations.

After submission of this paper it came to our attention that Keating¹³ has recently also suggested that the differing experimental results in *Rana* and *Xenopus* may relate to quantitative rather than qualitative differences between the two organisms.

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PAUL GROBSTEIN
CHRISTOPHER COMER

Department of Pharmacological
and Physiological Sciences,
University of Chicago
947 East 58th Street
Chicago, Illinois 60637

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Optimal mate selection in the toad *Bufo bufo*

SEXUAL reproduction can no longer be regarded as a cooperative venture in which males and females are selected to achieve the same goals. The optimal reproductive strategy for a male is often very different from that for a female^{1,2}. We report here that, in the wild, toads (*Bufo bufo*) do not pair up at random, that this comes about through male-male competition probably influenced by the behaviour of the female and that mating involves a compromise between different male and female optima.

Toads visit ponds to breed in early spring. All the year's reproductive activity takes place within a few weeks, after which the toads leave the pond and remain on land until the next year³. Our observations were made at a pond near Oxford in March and April 1977. Every female we found in the pond was already in amplexus; that is a male was clasped onto her back, gripping her pectoral girdle with his forearms. Males greatly outnumbered females, however,

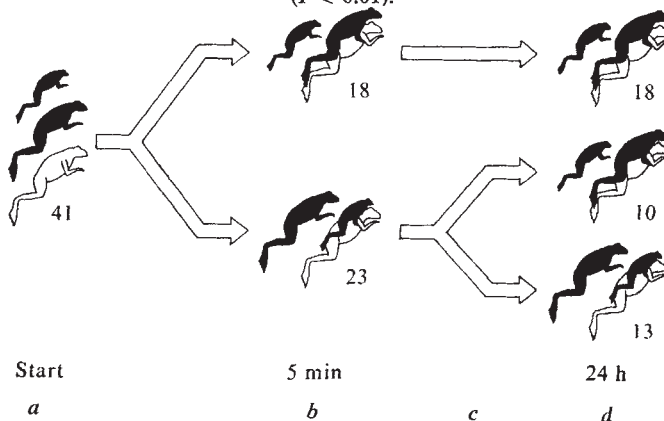
and during 11 d we found that $84.9 \pm 9.3\%$ (mean $\pm$ 1 s.d.) of the males were unpaired. The excess of males on any one day probably occurred because males can mate more than once and individuals spend most of the breeding season in the pond, whereas females, who can only spawn once, visit the pond for a shorter time. Thus at any particular time most of the male population is in the pond but only a small fraction of the female population^{3,4}.

We measured the snout-vent lengths of males and females we found in amplexus. Before the start of spawning there was no significant correlation between the length of males and females in amplexus (on one day, $r = 0.094$, $n = 32$; on another day, $r = 0.299$, $n = 26$). Small males were just as likely to be paired with large females as were large males. But when we measured the length of pairs actually engaged in spawning, we found a significant relationship ($r = 0.567$, $n = 16$, $P < 0.05$). By the time spawning had started the large females were paired with the large males. This presents two problems; first, what is the mechanism by which this non-random mating comes about and second, what is its functional significance?

What is the mechanism by which non-random mating comes about? In the laboratory we found that even males that were 30 mm smaller than their mates were easily able to maintain amplexus for a week or more. Thus the female herself is apparently unable to displace a male, even if he is very small. In the wild, however, we often found two males tussling over the possession of a female. To investigate the effect of male-male competition we performed a series of experiments in the laboratory, summarised in Fig. 1. The results showed that, just as in the wild, pairing was random at first but after some time large males were more likely to be paired with large females because they could achieve successful takeovers.

We conclude that male-male competition is the mechanism by which the change in pairing from prespawning to spawning comes about in the wild. Because it is the female who is in control of locomotion of the pair (her legs are in the most free position for swimming) it seems likely that she will be able to influence whether male-male competition takes place or not. If she has a suitable mate she may swim away from other males in order to avoid interference⁵. If she has been grabbed by an unsuitable male then we suggest that one strategy she could use would be to swim over to where there are other males and thus invite

Fig. 1 Male-male competition experiments in toads. Results of 41 experiments, each with one large male, one small male and a female in a tank. The experiments were done with various absolute sizes but in each, one male was larger than the other male by about 10 mm in body length. *a*, One female (white) and two males (black) are placed in a tank. *b*, Within a few minutes one of the males has paired up with the female. Either male is equally likely to be the first to adopt amplexus. *c*, The unpaired male attacks the pair and attempts to dislodge his rival. The paired male lashes out with his hind legs in defence. *d*, Large males can sometimes displace smaller males but not vice versa ($P < 0.01$).



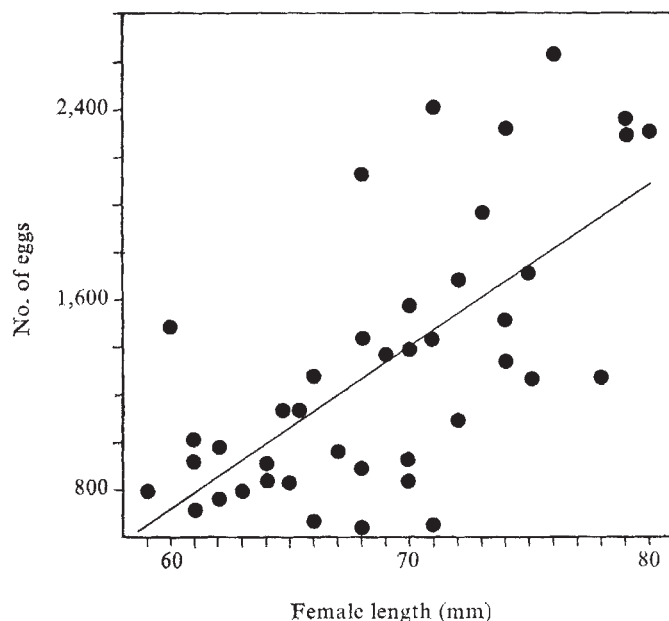


Fig. 2 Relationship between female body length, measured from snout to vent, and number of eggs laid. Results obtained from pairs spawning in individual tanks in captivity. A sample of 10 of the females was dissected and all had laid their full clutch of eggs. Fitted regression, $y = 67.47x - 3316.68$. A polynomial does not give a significantly better fit than the straight line. Correlation coefficient, $r = 0.669$, $P < 0.001$.

male-male competition. Obviously all this waste of time and energy could be avoided if the female found a suitable mate in the first place. In our laboratory experiments successful takeovers took on average 7 h to complete (mean $\pm$ 1 s.d. = 420.3 ± 321.8 min, $n = 10$). Although we have no evidence for our species, in many anurans the female uses the call of the male as a basis for mate choice⁶. In this context it is interesting that different sized males have different calls so it is possible that a female might select a male of suitable size on the basis of his call⁷.

What is the functional significance of the non-random mating found in the wild? To investigate this question we paired up toads in the laboratory with partners of a size that they would not have mated with in the wild, to see if reproductive success was thereby decreased. We measured reproductive success in terms of the number of fertilised eggs produced. For any given pair this will depend on the number of eggs laid by the female and the percentage of them that are successfully fertilised by the male. Figure 2 shows that, as in other amphibians⁸, the number of eggs laid by a female is related to her size; large females lay more eggs.

As with all cases of external fertilisation, the male has the problem of timing his release of sperm so that it coincides with the female's release of eggs. Precise synchrony of gamete release is presumably important for efficient fertilisation. In many anurans it seems that the stimulus for sperm emission is the passage of the extruding eggs over the venter, cloaca or feet of the male⁶. In some species, just before egg emission, the female arches her back to bring her cloaca in close contact with that of the male⁶.

Figure 3 shows that the degree of matching in size between male and female affects fertilisation success. Where the male is relatively small compared with the female the fertilisation success is lower than where the partners are matched for size. We suggest that the most likely explanation for this relationship is that relatively small males are inefficient at detecting egg release and thus synchronising their timing of emission of sperm. Alternatively where the cloaca of the male is not close to that

of the female, the sperm must have further to swim to reach the eggs and this may decrease fertilisation success.

From the relationship between female length and egg number (Fig. 2) and percentage fertilisation and relative size of male and female (Fig. 3), it is possible to calculate the optimum mate size for a male or female of any given size. To do this we assumed that what will be maximised is the number of fertilised eggs (tadpoles) produced per breeding season. For any given pairing this is simply the number of eggs (calculated from female body length) multiplied by the percentage of these eggs that are successfully fertilised (determined by the relative size of the male and female). To achieve maximum fertilisation of their eggs, females should prefer the largest males. Males, on the other hand, should prefer a different optimum. For any given sized male the number of fertilised eggs increases with female size up to a maximum and then declines. This is because with relatively small females he can achieve efficient fertilisation but small females produce only a few eggs, while at the other extreme, relatively large females lay more eggs but the male can only fertilise a small proportion of them. To maximise the number of fertilised eggs, males should prefer females about 10–20 mm larger than themselves.

Figure 4 shows that there is a conflict between the sexes in the optimal mate size for achieving the maximum number of fertilised eggs. The pairings we observed in the wild mostly fall in between the male optimum and the female optimum. Because females are on average larger than males, the points tend to fall closer to the male optimum. This, however, does not necessarily mean that the males are winning the battle of the sexes. The theoretical optima curves were calculated with the assumption that individuals will be selected to maximise the number of tadpoles produced per year. In long-lived species like toads, however, individuals may be prepared to sacrifice maximal yearly reproduction if by so doing they increase their chance of survival to breed again in future years^{9,10}. Indeed, offspring quantity may be an inappropriate measure of fitness; we have ignored the possible importance of offspring quality (as determined, for

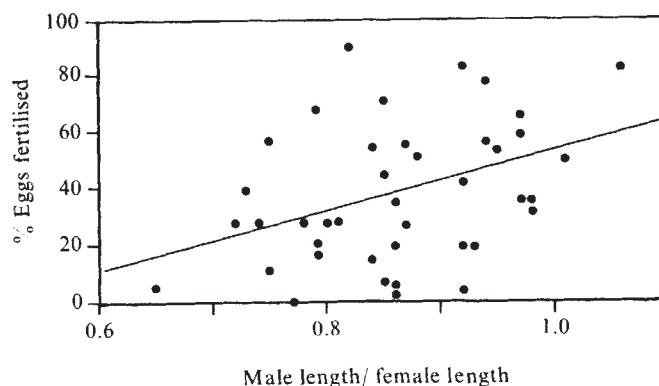


Fig. 3 The effect of relative male and female size (body length, snout to vent) in amplexing pairs of toads on the percentage of the eggs that are successfully fertilised. The smaller the male is relative to the female, the smaller the proportion of fertilised eggs. Pairs of toads were collected from the wild, parted and then formed into new pairs with various sizes of male and female in amplexus. Each pair was kept in a separate tank. The number of fertilised and unfertilised eggs were scored 15 d after egg laying. At this stage the fertilised eggs had developed into small tadpoles, while unfertilised eggs remained as unchanged spheres. None of 100 unchanged eggs examined microscopically from each pair had undergone any cell division. Fitted regression, $y = 104.73x - 52.36$. Correlation coefficient, $r = 0.379$, $P < 0.02$. When the effect of egg number on percentage fertilisation is partialled out, the effect of relative size is still significant ($P < 0.05$). Likewise, it is still significant ($P < 0.02$) when the effect of absolute size of male is partialled out.

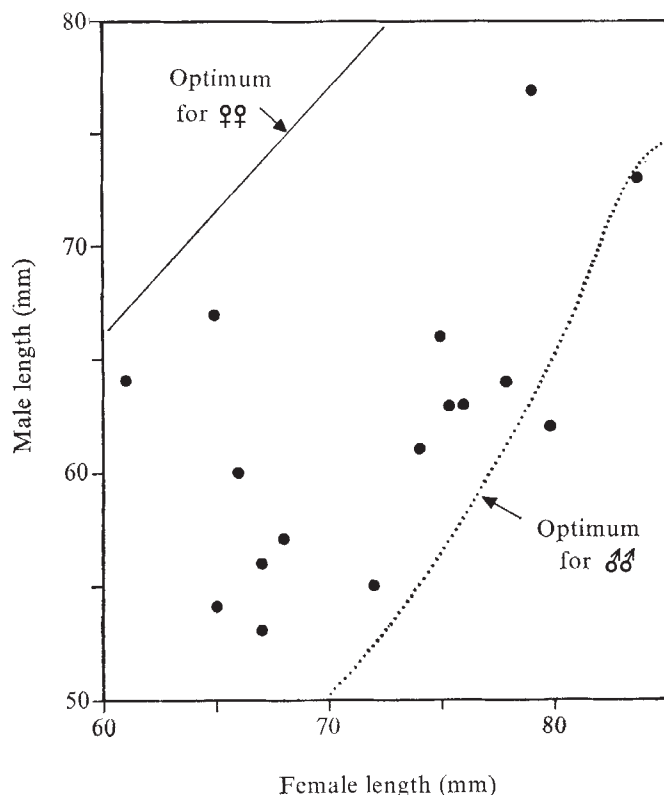


Fig. 4 The conflict between males and females in the optimum size of mate assuming that what is maximised is the number of fertilised eggs produced per year. The female line is a minimum because no experimental pairings were done with values greater than 1.1 (for male length divided by female length). If the line in Fig. 3 continued to rise with relatively larger males, then the female optimum curve should be shifted to the left so that larger males are preferred than is indicated. Observed pairings in the wild in the process of spawning (solid circles), fall in between the male and female optima. (Correlation coefficient, $r = 0.567$, $P < 0.05$.)

example, by egg size⁸). Finally we have examined mate selection solely with respect to body size whereas it seems certain that other characters will also influence fitness. In spite of these reservations, as far as we know these data provide the only clear evidence, apart from that from one laboratory study¹¹, that individuals can increase their fertility by non-random choice of mates.

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N. B. DAVIES
T. R. HALLIDAY

Edward Grey Institute and
Animal Behaviour Research Group,
Department of Zoology,
University of Oxford, UK

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Use of Ficoll-sodium metrizoate density gradient to separate human X- and Y-bearing spermatozoa

SEVERAL claims have been made about the successful separation of Y-bearing spermatozoa in the past¹. The most recent claim by Ericsson *et al.*² has been disputed^{3,4}. We have attempted to develop a method for separating human X- and Y-bearing spermatozoa, without affecting the motility or the variability of these vital cells. We report here a successful separation of human X- and Y-bearing spermatozoa using a two-step procedure with Ficoll-Sodium metrizoate density gradient. The motility and viability of enriched fractions of spermatozoa was found to be slightly reduced. The reduction was mainly due to centrifugation and not the density gradient. The ability to predetermine the sex of the offspring before conception would have great clinical and sociological significance⁵.

Semen was collected from normal human volunteers. Only samples with a count of over $6 \times 10^7 \text{ ml}^{-1}$ and a motility of at least 50% were used for the experiments. Experiments were performed within 1 h of sample collection.

Ficoll-400 (Pharmacia) and sodium metrizoate (Nyeguard) density gradients were used for the separation. Each semen sample was diluted 20-fold with phosphate buffered saline (PBS), pH 7.2 and centrifuged at 100g for 20 min. The spermatozoa were washed twice with the same buffer, in identical conditions.

In a one-step separation technique the washed spermatozoa were suspended in PBS and made up to the original volume of the semen. The density gradient was prepared by mixing 2.4 ml of 8% Ficoll with 1.0 ml of 32.8% sodium metrizoate. The density of this gradient was 1.08. The spermatozoa suspension was layered on Ficoll-sodium metrizoate density gradient and centrifuged for 20 min at 100g. After centrifugation different layers were separated and suspended in PBS, and the count adjusted to 10^6 ml^{-1} . Smears of these suspensions were stained for the detection of the fluorescent body.

In a two-step separation technique the first step separation was carried out as above. The enriched fractions obtained after the first step were again layered on 15% Ficoll of density 1.04. This was centrifuged for 10 min at 100g. The spermatozoa from the different layers were separated and smears were made for staining.

A modification of the method of Barlow and Vosa⁶ was used for staining the fluorescent body. The smears were air dried, fixed in methanol for 15 min stained by immersing the slides in 0.5% quinacrine hydrochloride (Sigma) for 15 min and washed in running tap water. Excess dye was removed by immersing the slide in PBS at pH 5.6, for 15 min. The slides were mounted in glycerolphosphate buffer (1:9), and examined under oil immersion with dark field condenser of Olympus fluorescent microscope with barrier filter Y-50 and exciter filter B₁. About 10 fields with a minimum of 100 spermatozoa were counted, from each slide, for the presence of the fluorescent body.

After centrifugation with Ficoll-sodium metrizoate it was seen that some of the spermatozoa had settled down and there was a very clear interface which was also rich in spermatozoa. The sediment (S) was rich in Y-bearing spermatozoa, whereas the interface consisted mostly of X spermatozoa (I). The mean percentage of Y-bearing spermatozoa was $43 \pm 2.7\%$ in unseparation (Table 1). The interface contained $74.7 \pm 2.9\%$ X spermatozoa, whereas the percentage in unseparated suspension spermatozoa, whereas the percentage in unseparated suspension was $56.3 \pm 2.7\%$.

In the two-step separation process the percentage of Y-bearing spermatozoa following the initial one-step separation (S_1) was $73.2 \pm 1.0\%$ and this increased only to $76.9 \pm 2.1\%$ after the second separation step (S_2) (Table 2). The results of the one and two step separation procedures with different samples were very consistent. Table 3 shows the percentages of X-spermatozoa before and after two-step separation. The mean percentage of X spermatozoa in the unseparated sample was 54.2 ± 3.2 . The first step procedure

Table 1 Percentages of X- and Y-bearing spermatozoa separated on Ficoll-sodium metrizoate density gradient (1.08) and centrifuged for 20 min

Experiment no.	% Y spermatozoa in washed samples before separation	% Y spermatozoa following separation (S)	% X spermatozoa before separation (I)	% X spermatozoa after separation
1	41.9	70.4	58.1	68.9
2	46.6	70.8	53.4	73.3
3	42.6	72.4	57.4	73.8
4	43.3	72.7	56.7	78.4
5	39.8	77.8	60.2	77.8
6	41.9	72.4	58.1	77.6
7	45.8	72.7	54.2	74.6
8	48.4	73.2	51.6	74.2
9	43.0	74.5	57.0	74.1
Mean $\pm$ s.d.	43.7 $\pm$ 2.7	72.8 $\pm$ 2.2	56.3 $\pm$ 2.7	74.7 $\pm$ 2.9

Spermatozoa were washed twice with PBS pH 7.2 before layering on density gradient.

yielded a fraction containing 74.7 ± 2.9 and the second step 80.6 ± 6.3 of X spermatozoa.

Our results demonstrate that the percentage of Y-bearing spermatozoa in fractions S_1 and S_2 and that of X spermatozoa in fractions I_1 and I_2 are considerably higher than in unseparated samples. It is known that spermatozoa in the semen of normal fertile human males, show considerable heterogeneity⁷. The X- and Y-bearing spermatozoa so separated seemed to be uniform in size and shape in each of the concerned fractions.

it may be assumed that the calculated percentage of the Y-spermatozoa in the one step procedure might be about 82% and in two-step separation it might be as high as 86%.

The Ficoll-sodium metrizoate density gradient has been used extensively for the separation of lymphocytes without any functional damage to cells⁹. Ficoll has also been used for washing spermatozoa¹⁰, and is known not to damage the cells. We have carried out a few experiments to check the motility and viability of spermatozoa following separation. We found that the original

Table 2 Percentage of Y-bearing spermatozoa following two-step separation

Experiment no.	% Y spermatozoa before separation	% Y spermatozoa following one-step separation (S_1)	% Y spermatozoa following two-step separation (S_2)
1	41.9	72.4	80.9
2	45.8	72.7	75.9
3	48.4	73.2	77.9
4	43.0	74.5	77.8
5	41.3	74.2	75.0
6	46.3	71.6	75.0
7	44.2	73.5	76.0
Mean $\pm$ s.d.	44.4 $\pm$ 2.6	73.2 $\pm$ 1.0	76.9 $\pm$ 2.1

The first separation (S_1) was carried out by layering washed spermatozoa on Ficoll-sodium metrizoate gradient of density 1.08. The spermatozoa thus separated were again layered on 15% Ficoll of density 1.04 (S_2).

It is of interest to note that the mean percentage of Y-bearing spermatozoa in semen samples used in the first set of experiments was $43.7 \pm 2.7\%$ and in the second set of experiments $44.4 \pm 2.6\%$. It is generally accepted that 50% of spermatozoa possess Y chromosomes. The lower percentage of Y-bearing spermatozoa observed by us could be due to the fading of fluorescence. It could also be that the ratio was low because of the difficulty of resolving fluorescent body which lies within the dense chromatin⁶. Yet, another reason may be the low uptake of the fluorescent dye by some spermatozoa⁸. If we take all these factors into consideration

motility (85%) was slightly reduced (75%) after the first centrifugation, and motility was further reduced to 58% after another centrifugation with PBS. But, when spermatozoa were layered on Ficoll-sodium metrizoate and centrifuged, motility was 65% and 70% in the interface and the sediment fractions respectively. Thus the gradient has a protective action on the cells, preserving sperm motility. Viability was slightly reduced after two centrifugations, however. This reduction in motility and viability is mainly due to centrifugation and not due to the effect of density gradient. It has been reported¹¹ that following artificial insemination (AI) there

Table 3 Percentage of X-bearing spermatozoa following two-step separation

Experiment no.	% X spermatozoa before separation	% X spermatozoa following one step separation (I_1)	% X spermatozoa following two-step separation (I_2)
1	58.1	77.6	94.4
2	54.2	74.6	80.5
3	51.6	74.2	76.5
4	57.0	74.1	76.6
5	48.7	78.8	78.8
6	53.7	69.8	77.2
7	55.8	73.5	80.2
Mean $\pm$ s.d.	54.2 $\pm$ 3.2	74.7 $\pm$ 2.9	80.6 $\pm$ 6.3

The first separation (I_1) was carried out by layering washed spermatozoa on Ficoll-sodium metrizoate gradient of density 1.08. Spermatozoa thus separated were again layered on 15% of Ficoll of density 1.04 (I_2).

was an increased number of female offsprings when insemination was carried out 3–4 days before ovulation and an increased probability of males as the ovulation approached. The probability of producing offspring of a desired sex would be enhanced if AI was carried out at the appropriate time¹¹.

PADMA R. SHASTRY
UMASHASHI C. HEGDE
SHANTA S. RAO

*Institute for Research in Reproduction,
Jehangir Merwanji Street, Parel,
Bombay 400 012, India*

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Antigen expression on early mouse trophoblast

THE mammalian foetus inherits paternal as well as maternal genes and can be considered to constitute a naturally occurring and highly successful allograft¹. It has been suggested that an important factor in the survival of the foetus in an immunocompetent histoincompatible mother might be the presence of an antigenically deficient barrier of foetal trophoblast cells^{2–4}. Cell surface antigens determined by minor (non-H-2) histocompatibility loci have been demonstrated on cleavage stage mouse embryos⁵, and subsequently major (H-2) as well as non-H-2 determinants have been detected on the trophectoderm of pre-implantation mouse blastocysts^{6,7}. The expression of these antigens, however, is considerably reduced, below detectable levels in the case of H-2, on blastocytes which have been activated for implantation from a state of experimentally induced delay^{6,7}. In addition, immunofluorescence⁸, immunoperoxidase⁷ and mixed haemadsorption (MHA)⁹ studies have failed to demonstrate histocompatibility antigens on the trophoblast component of blastocysts cultured as outgrowths *in vitro*. In contrast to all these findings, however, a recent report has claimed to demonstrate both maternally and paternally inherited antigens on blastocyst outgrowths by means of an MHA technique¹⁰. We have now confirmed this report and reconciled the apparently contradictory results by investigation of the identity of the antigens expressed by the trophoblast at this early stage of its differentiation in four mouse inbred strains.

Blastocysts from spontaneously ovulating females were collected approximately 80 h after mating by flushing the excised uteri with phosphate-buffered saline (PBS). After removal of the zona pellucida by digestion with 0.5% Pronase, embryos were cultured in groups of four or five in the wells of plastic migration plates (Sterilin) under RPMI 1640 medium. MHA tests were performed after 96 h in culture. Blastocyst outgrowths were incubated for 2 h with various dilutions of specific alloantisera either obtained commercially (Searle Diagnostic) or raised between inbred strains of mice by sequential intraperitoneal injections of adult spleen cells. After thorough washing with PBS, a 0.5% suspension of freshly prepared indicator sheep red cells (ISRC)¹¹ was added to the cultures and incubation continued for a further 1 h. Cultures were finally washed in PBS and examined by phase contrast microscopy for adherence of ISRC. Scoring was based on a visual assessment similar to that described by Hausman and Palm¹² and was confirmed by an independent observer. The outgrowths were subsequently fixed in 2.5% glutaraldehyde and stained with

Giemsa as a permanent record. The results are summarised in Table 1.

Although both CBA and C57BL outgrowths showed strongly positive adherence of ISRC after incubations with antisera raised against multiple (H-2+non-H-2) or minor (non-H-2) specificities, no adherence was noted when antisera raised between strains congenic for H-2 were used. The results imply that the reactivity previously reported with these strains¹⁰ is not due to antigens specified by the major histocompatibility complex (MHC). Similar adherence was not observed on A outgrowths when tested with an antiserum against multiple specificities raised in C57BL mice, thus confirming earlier results using this strain combination^{9,13}.

CBA anti-C57BL antiserum detects non-H-2 antigens on B10.BR outgrowths, which is probably a reflection of the common genetic background of the C57BL and B10.BR strains¹⁴, and also detects non-H-2 antigens on A strain trophoblast. Non-H-2 antigens, however, cannot be detected on A outgrowths by either C57BL anti-A or C57BL anti-CBA antisera, even though the latter, adsorbed to remove activity against H-2 antigens, is strongly positive in MHA tests against monolayer cultures of A strain embryonic fibroblasts (unpublished observations). Conversely, the C57BL anti-A antiserum, which is negative for A outgrowths, gives a weakly positive reaction with CBA outgrowths, implying that non-H-2 antigens expressed by adult A strain spleen cells and shared by CBA outgrowths are nevertheless not expressed on A strain trophoblast. This reactivity is unlikely to be a result of shared H-2^k specificities since CBA outgrowths do not react with the congenic anti-H-2^k serum. Since outgrowth from F₁ hybrid blastocysts of reciprocal CBA×A matings continue to express CBA-associated antigens, of both maternal and paternal origin (unpublished observations), and A outgrowths express non-H-2 antigens detected by CBA anti-C57BL antiserum, it is unlikely that any gross masking effect is responsible for the non-expression on A outgrowths of those non-H-2 antigens which are shared by CBA trophoblast and adult A strain tissues. It is therefore clear that H-2 antigens are not expressed on trophoblast at this stage of development and also that there are strain differences in the expression of non-H-2 antigens.

It has been recognised that determinants controlled by the MHC are involved in the interaction of T lymphocytes

Table 1 Detection of cell surface antigens on trophoblast of blastocyst outgrowths by mixed haemadsorption assay (MHA)

Antiserum	Antigens detected	CBA (H-2k)	Target outgrowth C57BL (H-2b)	A (H-2kd)	B10.BR (H-2k)
C57BL anti-CBA	H-2k+non-H-2	+++	—	—	—
CBA anti-C57BL	H-2b+non-H-2	—	+++	++	++
C57BL anti-A	H-2kd+non-H-2	+	—	—	ND
C57BL/10ScSn anti-B10.BR	H-2k	—	—	—	—
B10.BR anti-C57BL/10ScSn	H-2b	—	—	—	—
C57BL anti-CBA (abs)*	non-H-2 only	+++	ND	—	ND

+++ , Heavy adsorption of ISRC on all trophoblast cells. ++ , moderate adsorption of ISRC on > 80% of trophoblast cells. + , light adsorption of ISRC on > 50% of trophoblast cells. — , no adsorption of ISRC. (Occasionally one or two cells in some outgrowths showed strong adsorption. These were very likely of inner cell mass origin.) ND, not determined. Each result is based on observation of not less than 10 outgrowths.

*C57BL anti-CBA antiserum was adsorbed twice against equal volumes of washed B10.BR lymphoid cells. Each adsorption was for 1 h at 37 °C and the resulting serum had no residual activity against H-2k as measured by MHA on B10.BR embryonic fibroblasts.

with non-MHC antigens¹⁵. Lack of reactivity of blastocyst outgrowth trophoblast with antisera which are probably directed against the products of the entire MHC may imply an absence not only of those determinants which are necessary for effector recognition by cytotoxic T lymphocytes (K and D region determinants)¹⁶ but also of those which are necessary for T-cell sensitisation (the I region associated Ia antigens)¹⁷. Thus the absence of MHC products may enable the trophoblast to fulfil an immunological barrier function because it is unlikely that the expression of non-MHC antigens could render it susceptible to damage by maternal T cells. The possible interplay between these components of the trophoblast cell surface and other types of maternal immune response as well as their potential involvement in developmental interactions with maternal and/or foetal cells is unexplored.

It must be emphasised that these findings relate only to the trophoblast of the blastocyst outgrowth, for the antigenic status of trophoblast is not constant throughout its ontogeny and differentiation^{9,13}. This probably reflects a changing functional role for this tissue in the materno-foetal immunological relationship.

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M. H. SELLENS

Reproductive Immunology Research Group,
Department of Pathology,
The Medical School, University of Bristol,
Bristol, UK

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Ly-6 is a T-cell differentiation antigen

THERE is ample evidence that membrane antigenic markers change as the cells differentiate along a given pathway. Thus a B cell acquires its receptors, immunoglobulin, as it matures to a functional state. Yet other membrane markers are acquired as cells differentiate from precursor to the activated effector cell stage. For example, the membrane alloantigen Pca-1 is acquired as B cells become antibody-forming cells¹. Another example is the alloantigen Ala-1, presented on activated lymphocytes of both T- and B-cell series, but not on resting cells². Suppressor cells are lysed by anti-Ia antisera³ while their precursors are not⁴, suggesting either the acquisition of Ia antigens on activation, or a change in susceptibility to lysis. This is of interest as the active molecules of suppression, 'suppressor factors', themselves bear Ia antigens⁵. Thus, in this instance there is the acquisition of a membrane marker which is also present in the functional effector molecule. McKenzie and his colleagues recently described a new T-cell alloantigen, Ly-6, which is expressed on the majority of peripheral T cells but only on a small proportion of thymocytes⁶. Using antiserum directed against Ly-6.2 we have published evidence suggesting that this antigen is present on cytotoxic effector cells⁷.

In the work presented here we extend those investigations, showing that while Ly-6 is present on cytotoxic effector cells it is absent from precursor cells, unlike the Ly-1, 2, 3 antigens where precursor cells usually carry the same antigens as effectors⁸. These observations suggest that Ly-6 can be considered as a unique T cell-restricted differentiation antigen that evolves fairly late in the maturation scheme, at least for killer cells.

When various antisera, directed against cell surface allo-antigens were used to phenotype the cytotoxic effector cells (Fig. 1), we found that AKR (Ly-1.2) killer cells express the phenotype Thy-1⁺ Ly-1.2⁺3⁺5⁺6⁺, although in other studies using Ly-1.1 positive strains, such as CBA, killer cells bear the phenotype Thy-1⁺ Ly-1.2⁺3⁺ (refs 2, 9). To study the phenotype of the precursor cell it was necessary to treat normal spleen cells with the various antisera and complement and then examine the ability of the remaining cells to generate cytotoxic effector cells. The results of three such experiments are shown in Table 1. The data on total activity indicate that the reduction in killing capacity seen in the populations treated with anti-Ly-2.1 or anti-Thy-1.1 is not the result of poor culture viability. It is clear that the phenotype of the killer cell precursor is Thy-1⁺ Ly-1.2⁺6⁻ indicating that the alloantigen Ly-6 is either not carried on the precursor cells, or that the amount of antigen present is not sufficient to mediate complement dependent lysis. A third possibility would be that these particular cells are resistant to complement lysis, however, this is unlikely as the cells are susceptible to lysis by other alloantisera of the same titre such as anti-Thy-1 and anti-Ly-2. In two experiments (data not shown) the anti-Ly-6 was used two- and fourfold more concentrated than necessary to have plateau level killing. Results of these assays were similar to those shown. Treatment, however, of normal cell populations using alloantisera at plateau levels of cytotoxicity fails to eliminate

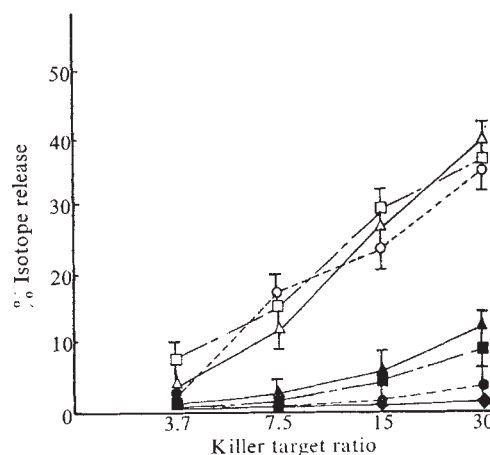


Fig. 1 Treatment of cytotoxic effector cells with various allo-antisera and complement. AKR spleen cells (H-2^k, Thy-1.1, Ly-1.2, Ly-2.1, Ly-5.1, Ly-6.2) at 10×10^6 per ml were incubated with 3×10^6 irradiated (2,000 rad) BALB/c (H-2^d) spleen cells for 6 d in Marbrook tissue culture flasks. The cells were collected and aliquots placed in tubes containing 3×10^6 viable cells. The cells were treated with 0.3 ml of alloantiserum, used at plateau levels of cytotoxicity, for 30 min at 37 °C, washed, and then treated with 0.3 ml complement diluted to 10%, controls included cells treated with media or complement only. The treated cells were then resuspended to 0.5 ml and serially diluted in microtitre plates. Chromium labelled P815 (H-2^d) mastocytoma cells were used as targets, and were added such that the highest killer target ratio was between 20 : 1 and 30 : 1. After initial centrifugation at 200g the assay was incubated for 4 h at 37 °C, centrifuged again, and 100 µl of supernatant aspirated for counting. The % isotope release is calculated as for Table 1. The data are expressed as the mean % isotope release of duplicate values $\pm$ s.e.m. Antisera— Δ Ly-1.2, $\square$ Ly-2.1, $\circ$ Ly-5.1, $\blacksquare$ Ly-6.2, $\blacklozenge$ Thy-1.1.

Table 1 Effect of pretreatment of normal spleen cells with alloantiserum and complement on ability to generate cytotoxic effector cells

Treatment of normal spleen cells	Experiment 1	% Isotope release (total activity) Experiment 2	Experiment 3
Nil	90±0.5 (1,260)	60±3 (1,020)	70±5 (1,008)
C' alone	83±3.0 (1,162)	58±2 (986)	60±1 (678)
Anti-Ly-1.2+C'	81±2.0 (1,134)	70±4 (1,470)	ND
Anti-Ly-2.1+C'	52±1.0 (899)	20±2 (300)	ND
Anti-Ly-6.2+C'	84±1.0 (1,130)	54±3 (1,080)	57±1 (974)
Anti-Thy-1.1+C'	48±1.0 (614)	18±3 (270)	16±1 (142)

30×10^6 normal AKR spleen cells (H-2^k, Thy-1.1, Ly-1.2, 2.1, 6.2) were treated with various alloantisera, used at plateau levels of cytotoxicity, and rabbit serum, absorbed with mouse spleen cells, as a source of complement. The cells remaining after a two-stage cytotoxicity procedure were adjusted to 10×10^6 cells per ml and incubated with 3×10^6 irradiated (2,000 rad) BALB/c (H-2^d) spleen cells, for 6 d in a Marbrook tissue culture chamber. The cells were collected and tested for their ability to lyse chromium labelled P815 (H-2^d) mastocytoma cells in a 4-h assay. A peak killer target ratio of 20 : 1 was used, with serial dilutions of the effector cells (not shown). The data in each experiment shown for the 20 : 1 ratio are also valid for each of the serial dilutions. The % isotope release was calculated using the formula

$$\% \text{ isotope release} = \frac{\text{Experimental value} - \text{medium background}}{\text{Detergent release} - \text{medium background}} \times 100$$

Total activity = % viable cell yield $\times$ % isotope release at 20 : 1 K:T ratio. Values are mean $\pm$ s.e.m. ND, Not determined.

all of the precursor cells. This is not surprising as the actual precursors are likely to be heterogeneous with regard to the amount of antigen expressed on the cell surface and as some might escape antiserum treatment. This might be expected in the case of weak alloantisera where the effective antibody is in low concentration. An alternative view is that if only a small percentage of precursors escape kill these may still give a surprisingly high response because feedback controls provided by the missing cells are now lacking.

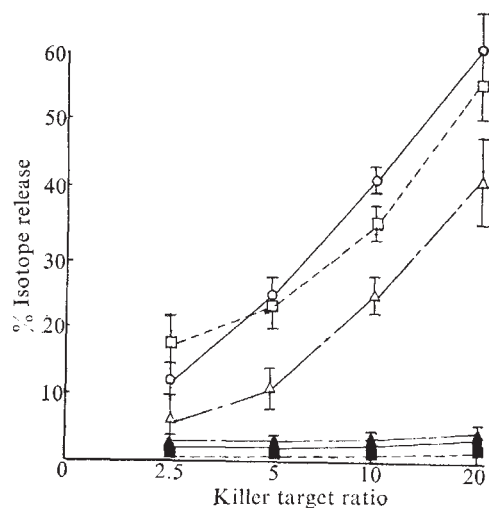
Figure 2 shows the results of experiments in which cytotoxic effector cells were generated from anti-Ly-6.2 treated spleen cells. The killer cells were then retested for phenotype using the various alloantisera. It is clear that the phenotype Thy-1⁺ Ly-1⁻2⁺6⁺ is identical to killer cells generated from untreated spleen cells. This suggests that only one pathway is open for the maturation of killer cells, and that at least for the allogeneic system, only one type of cytotoxic cell can be generated in that they are all Ly-6⁺. Replicate experiments have given similar results.

Mouse strains used for producing anti-Ly-6.2 also differ at Ala-1, hence a theoretical possibility exists that Ala-1.2

contaminates our anti-Ly-6.2, although the former alloantisera is produced by immunisation with mitogen-activated cells, and the latter with resting spleen and lymph node cells, which should not carry the Ala-1 antigens. The results of an experiment designed to answer this question are shown in Table 2. After five absorptions of anti-Ly-6.2 with 129 mouse spleen cells (Ly-6.2, Ala-1.1) the ability to lyse killer cells was essentially gone indicating that the major cytotoxic activity of anti-Ly-6.2 is directed against that alloantigen, and that any anti-Ala activity present is at best very weak. The reduction of activity in anti-Ly-6 absorbed with CBA spleen cells probably represents some dilution as well as passive absorption, to be expected when multiple absorptions are performed.

In addition to the implications with regard to the diversity of T cells, our findings suggest that Ly-6 is a differentiation antigen for cytotoxic effector cells. Whether this holds true for other functional T cells such as suppressor cells and helper cells is under study. If Ly-6 is a differentiation antigen for effector T cells one might wonder why it is cytotoxic for such a large number of peripheral T cells. It could well be that the majority of peripheral cells are in fact memory cells for helper, suppressor or killer populations awaiting reactivation with antigen and hence might be susceptible to Ly-6 treatment. One might predict that Ly-1⁺ helper cells¹⁰, Ly-2⁺3⁺ suppressor cells¹¹ and Ly-1⁺2⁺3⁺ killer cells¹², will be sensitive to anti-Ly-6.2 in contrast to their precursors.

Fig. 2 Treatment of cytotoxic effector cells generated from anti-Ly-6.2 treated spleen with various alloantisera. 120×10^6 normal AKR (H-2^k, Thy-1.1, Ly-1.2, Ly-2.1, Ly-6.2) spleen cells were treated with anti-Ly-6.2 and complement. The viable cells (10×10^6 per ml) were incubated with 3×10^6 per ml irradiated (2,000 rad) BALB/c (H-2^d) spleen cells for 6 d in Marbrook tissue culture flasks. The cells were collected and aliquots placed in tubes containing 2×10^6 viable cells. Treatment with the various alloantisera and assay for cytotoxic activity were as for Fig. 1. Symbols as in Fig. 1.

**Table 2** Ability of anti-Ly-6.2 to lyse cytotoxic effector cells after absorption with spleen cells from various mouse strains

Alloantiserum	Spleen cell absorption	C'	(Ly-6.2 ⁺ Ala-1.2 ⁺) effector cells	% Isotope release
—	—	—	3×10^6	52±5*
—	—	+	3×10^6	53±1*
Anti-Ly-6.2	—	+	3×10^6	1±1
Anti-Ly-6.2	CBA (Ly-6.1, Ala-1.1)	+	3×10^6	30±1
Anti-Ly-6.2	C57BL (Ly-6.2, Ala-1.2)	+	3×10^6	52±1*
Anti-Ly-6.2	129 (Ly-6.2, Ala-1.1)	+	3×10^6	44±1*
Anti-Thy-1.1	—	+	3×10^6	1±1

Aliquots of 0.5 ml of a 1/10 dilution of anti-Ly-6.2 were absorbed five times with packed spleen from various mice. Each absorption used 10 volume % packed spleen cells, incubating with the antiserum for 45 min at 37°C, followed by centrifugation to remove the cells and repeating the process. After the final absorption each serum was incubated with 3×10^6 cytotoxic C57BL (H-2^b, Ly-6.2, Ala-1.2) effector cells for 30 min at 37°C. The cells were washed and incubated for a further 45 min with 0.5 ml of spleen absorbed rabbit complement diluted to 10%. After washing, the cells were resuspended to equal volumes and serially diluted in microtitre plates. The assay for cytotoxic activity was identical to that described in Fig. 1.

*Indicates that samples are not significantly different ($P > 0.05$).

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JAMES N. WOODY*

ICRF Tumour Immunology Unit,
Department of Zoology,
University College London,
Gower Street, London WC1, UK

Received 8 June; accepted 7 July 1977.

* Present address: Cellular Immunology Division, Naval Medical Research Institute, Bethesda, Maryland 20014.

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Regulation of requirements for anchorage-independent growth of Syrian hamster fibroblasts by somatic mutation

THE ability of mammalian cells to form colonies following suspension in semisolid agar, termed 'anchorage independence', is a phenotypic characteristic associated with cells transformed by oncogenic viruses and chemical carcinogens^{1–5}. Because this phenotypic characteristic has been correlated with tumorigenicity^{2–5}, colony formation in semisolid agar is used frequently as a quantitative *in vitro* assay for neoplastic transformation, especially in studies in Syrian hamster cell systems^{4,5}. It is therefore important to understand the molecular and physiological basis of this phenotype. If the expression of this phenotype can be altered by somatic mutation of identifiable genes, information can be gained concerning the mechanism of anchorage-independent growth *in vitro* and its exact relationship to tumorigenicity and malignancy *in vivo*. We have investigated the ability of cells of the highly tumorigenic Syrian hamster fibroblast line, BP6T (ref. 9) to form colonies when suspended in semisolid agar. Cells of this line produce fibrosarcomas in 100% of newborn Syrian hamster inoculated with ten cells. In addition to anchorage-independent growth, BP6T cells also exhibit other *in vitro* characteristics of neoplastic transformed cell lines, such as increased plating efficiency in liquid medium at low cell densities, and enhanced fibrinolytic activity⁹. In this communication, we report that the ability of this Syrian hamster line to form colonies in semisolid agar is dependent on its utilisation of exogenous purines by way of the hypoxanthine phosphoribosyltransferase (HPRT, E.C.2.4.2.8.)- and adenine phosphoribosyltransferase (APRT, E.C.2.4.2.7.)-mediated salvage pathways. Furthermore, this anchorage-independent property can be inhibited by a somatic mutation at the HPRT locus.

Supplementation of semisolid agar medium with either bactopectone or bactotryptose is a routine procedure when semisolid agar is used as a test medium for anchorage-independent growth^{1–6}. We found that in the absence of bactopectone, the ability of BP6T to produce colonies in semisolid agar was greatly influenced by the density at which the cells were suspended in the agar-containing

medium. At plating densities of less than 10⁴ cells per plate, colony formation was less than 0.1%. As shown in Table 1, however, supplementation of the agar suspension with 0.12% bactopectone permitted a colony-forming efficiency of 80% when 10–100 total cells were plated in each dish. With bactopectone, when the plating inoculum was increased above 10² cells, the efficiency of colony formation declined, decreasing to a value of approximately 30% at an inoculum of 10³ cells (Table 1). This reduction in the efficiency of colony formation with increasing cell density may have resulted both from the convergence of colonies and the competition for growth factors contained in the foetal calf serum and bactopectone supplements.

Since bactopectone has been reported to contain both hypoxanthine, and the unusual purine, 6,8-dihydroxypurine^{12,13}, we examined the ability of hypoxanthine and other purines to replace the bactopectone requirement. Table 2 demonstrates that hypoxanthine (4 × 10⁻⁵ M) was effective in permitting colony-forming efficiency of 30–42%, whereas guanine (4 × 10⁻⁵ M), was only partially effective, giving a colony-forming efficiency of approximately 20%. Xanthine, which is an inefficient substrate for hamster HPRT (ref. 7), did not support colony formation in agar. These results indicated that hypoxanthine or guanine could replace the bactopectone requirement for efficient colony formation in semisolid agar.

Since the utilisation of hypoxanthine and guanine is catalysed by the salvage enzyme HPRT, we examined the ability of several independently isolated HPRT-deficient strains of BP6T to form colonies in bactopectone-supplemented agar. Three spontaneously occurring (subclones 1–3) and three N-methyl-N'-nitro-N-nitrosoguanidine-induced (subclones 4–6) HPRT-deficient colonies were isolated from BP6T following independent selections in 1–5 µg ml⁻¹ 6-thioguanine. Each of these subclones of BP6T contained less than 1% of the parental HPRT activity, as assayed by the ability of intact cells to incorporate ¹⁴C-hypoxanthine into an acid-precipitable form¹⁰. Moreover, these strains maintained their HPRT-deficient phenotype for at least 40–50 population doublings in the absence of 6-thioguanine, with a reversion frequency less

Table 1 Effect of cell density and bactopectone supplement on colony formation efficiency in semisolid agar

Condition	BP6T cell number seeded	Colony number	Colony formation efficiency
With bactopectone 0.12%	10	9	90
	100	80	80
	200	103	50
	500	186	37
	1,000	260	26
Without bactopectone	100	0	0
	1,000	0	0
	10,000	> 300	> 3

The cell culture medium used was IBR modified Dulbecco's Eagle reinforced medium (ERM) (Biobabs, Northbrook, Illinois) supplemented with 0.22 g% NaHCO₃ and 10% Rehatuin F.S. foetal bovine serum (RFS) (Reheis Chemical, Kankakee, Illinois) without antimicrobial agents. Cells were transferred by a gentle trypsinisation with 0.1% trypsin solution (1:250, Gibco, Grand Island, New York) for 5 min at 36.5 °C. Agar cultures contained 3 ml base agar (0.6% agar dissolved in the above tissue culture medium) and 4 ml semisolid agar (0.3–0.4% agar dissolved tissue culture medium) in 35-mm or 60-mm diameter plastic Petri dishes. Before suspension in agar, cells were trypsinised and diluted into tissue culture medium. The cell number in the suspension was counted using a Coulter counter; a pre-determined number of cells was then suspended in the semisolid agar solution at temperatures between 37 and 40 °C, and the agar solution poured just before solidification. Agar cultures were incubated in a 37 °C incubator in a 5% CO₂ atmosphere at 90% relative humidity. At the end of 12–14 d, the number of colonies was counted under a light microscope. Each assay condition was measured in triplicate and the averaged results reported.

Table 2 Effect of purines on colony formation efficiency in semisolid agar by wild-type HPRT⁻ strains of BP6T cells

Cell strain	Agar supplement	Cell number seeded	Colony number	Colony formation efficiency (%)
BP6T (wild-type)	None	1,000	0	0
	Hypoxanthine 4×10^{-5} M	250	106	42
	Hypoxanthine 4×10^{-5} M	600	223	37
	Hypoxanthine 4×10^{-5} M	1,000	> 300	> 30
	Guanine 4×10^{-5} M	500	96	19
	Guanine 4×10^{-5} M	1,000	212	21
	Xanthine 4×10^{-5} M	500	0	0
	Xanthine 4×10^{-5} M	1,000	2	< 1
	Adenine 4×10^{-5} M	500	234	47
	Adenine 4×10^{-5} M	1,000	300	> 30
BP6T subclone 4 (HPRT ⁻)	Bactopeptone 0.12%	500	0	0
	Bactopeptone 0.12%	1,000	0	0
	Hypoxanthine 4×10^{-5} M	500	0	0
	Hypoxanthine 4×10^{-5} M	1,000	0	0
	Adenine 4×10^{-5} M	500	159	32
	Adenine 4×10^{-5} M	1,000	254	25
BP6T subclone 5 (HPRT ⁻)	Bactopeptone 0.12%	550	0	0
	Bactopeptone 0.12%	1,000	< 1	< 1
	Bactopeptone 0.12% + adenine 4×10^{-5} M	550	142	26
	+ adenine 4×10^{-5} M	1,000	268	27

than 2×10^{-7} (as measured by their sensitivity to medium containing hypoxanthine $13.6 \mu\text{g ml}^{-1}$, amethopterin $0.19 \mu\text{g ml}^{-1}$ and thymidine $3.9 \mu\text{g ml}^{-1}$ (ref. 11).

The ability of these HPRT-deficient strains of BP6T to form colonies in bactopeptone-supplemented agar (Table 3) was either greatly diminished or totally lacking at

colonies (subclones 2, 3 and 6). This represents minimally a 50–300-fold decrease in colony-forming efficiency in the HPRT-deficient strains compared with the wild-type BP6T strain.

When the wild-type BP6T and the HPRT-deficient strains were assayed by the same procedure¹⁰ for ¹⁴C-adenine incorporation by way of the APRT pathway, all the HPRT-deficient strains incorporated adenine into acid-precipitable material at a level comparable with the parental BP6T wild-type strain. This result indicated that the HPRT-deficient strains possessed a functional adenine salvage pathway. Since HPRT-deficient strains of BP6T cells retained the ability to salvage adenine and consequently the ability to convert AMP to other purine nucleotides (IMP and GMP), we examined the ability of adenine to support colony formation by these deficient cells in bactopeptone-supplemented agar. Table 2 shows that HPRT-deficient strains (subclones 4 and 5), which did not form colonies in semisolid agar supplemented with either bactopeptone or hypoxanthine, regained the ability to form colonies in agar at 24–34% efficiency after the addition of adenine 4×10^{-5} M). Adenine was also found to support colony formation by the wild-type BP6T strain in unsupplemented agar, as a replacement for bactopeptone (Table 2).

These results indicate that the malignant Syrian hamster fibroblast cell line, BP6T, requires exogenous purines and appropriate functional salvage enzymes to form colonies efficiently in semisolid agar. Other *in vitro* properties of this cell line, such as cloning efficiency in liquid medium and enhanced fibrinolytic activity were essentially

Table 3 Effect of HPRT deficiency on colony formation in semisolid agar*

BP6T strain	Cell number seeded	Colony number	Colony formation efficiency %
BP6T (wild type)	200	103	50
	500	186	37
	1,000	260	26
BP6T subclone 1 (HPRT ⁻)	500	0	0
BP6T subclone 2 (HPRT ⁻)	550	6	1
BP6T subclone 3 (HPRT ⁻)	500	3	< 1
BP6T subclone 4 (HPRT ⁻)	1,000	1	< 1
BP6T subclone 5 (HPRT ⁻)	1,000	1	< 1
BP6T subclone 6 (HPRT ⁻)	1,000	11	1

* The semisolid agar media were all supplemented with bactopeptone, 0.12%.

plating densities of less than 10^4 cells. After 14 d incubation, 200–500 BP6T cells produced colonies at an efficiency of 30–50% (Table 3), whereas 500–1,000 HPRT-deficient cells from six individual clones produced either one or no colonies (subclones 1, 4 and 5) or from three to eleven

Table 4 Summary of *in vitro* properties of Syrian hamster cell lines

Cell type	Genotype	CE*	Fibrinolysis†	Colony-forming efficiency‡ in semisolid agar	Tumorigenicity§
Syrian hamster embryo cells	HPRT ⁺	2–6%	(–)	0%	–
BP6T	HPRT ⁺	80%	+	50–80%	+
BP6T subclone 5	HPRT ⁻	41%	+	< 1%	+
BP6T subclone 6	HPRT ⁻	61%	+	1%	ND

* CE is cloning efficiency in liquid medium.

† Fibrinolytic activity was measured both in the extracellular medium and in cell lysates by procedures described previously⁹.

‡ The semisolid agar media were supplemented with 0.12% bactopeptone only. Values for the BP6T derivatives were taken from Table 3.

§ Syrian hamster embryo cells exhibited no colonies when 10^6 cells were tested.

§ Tumorigenicity was measured by subcutaneously injecting 0.1 ml complete medium containing varying concentrations of cells into non-immunosuppressed neonatal littermates (1–3-d-old) of outbred Syrian hamsters (Lakeview). Injection of 10^7 Syrian hamster embryo cells produced no tumours, whereas injection of 10^2 cells of BP6T and BP6T subclone 5 produced tumours.

unaltered by the forward mutation at the HPRT locus (Table 4). Additionally, the population-doubling times and cell saturation densities were unchanged (data not shown). It would be interesting to revert HPRT-deficient strains to HPRT⁺ to see if growth in agar supplemented only with hypoxanthine is regained. To date, however, attempts to isolate HPRT⁺ revertants of these strains have been unsuccessful.

Previous studies have shown that virally transformed derivatives of BHK 21 clone 13 (an established cell line of Syrian hamster origin) also require exogenous purines to support colony formation in agar, although not in liquid medium^{6,7}. Furthermore, 'normal' BHK cells (which induce tumours with high numbers of injected cells⁸) can be stimulated to form colonies in semisolid agar if thymidine is supplied in addition to the required purine supplement⁸. In contrast to our findings with BP6T, we observed no dependence on purines or purine salvage enzymes for anchorage-independent growth by HPRT- and APRT-deficient mouse fibroblast strains (data not shown). This finding suggests that *in vitro* requirements for anchorage-independent growth may vary between cells of different species origin.

Recently, stable variants which have lost certain *in vitro* phenotypes often associated with neoplasia have been isolated after treatment with mutagens^{4,14,15}. Such studies frequently rely on assumed correlations between demonstrated cellular alterations *in vitro* and tumorigenicity *in vivo*. Our results demonstrate that somatic mutation in tumorigenic Syrian hamster cells can lead to altered anchorage requirements for cellular growth, although the tumorigenic potential of these cells need not be lost (Table 4). Therefore, alterations in requirements for anchorage-independent growth *in vitro* may not necessarily correlate with malignancy.

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J. C. LEAVITT
B. D. CRAWFORD
J. C. BARRETT
P. O. P. TS'O

Department of Biochemical and Biophysical Sciences,
School of Hygiene and Public Health,
Johns Hopkins University,
Baltimore, Maryland 21205

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Rapid induction of amphotericin B sensitivity in L1210 leukaemia cells by liposomes containing ergosterol

AMPHOTERICIN B is a polyene antibiotic whose clinical use has been limited to the treatment of fungal infections. The mode of action of amphotericin B is believed to involve damage to cell membranes containing certain sterols^{1,2}. One sterol of primary importance for this membrane interaction is ergosterol²⁻⁵, and it is believed that its presence in

cell membranes of fungi confers sensitivity to amphotericin B². Studies with phospholipid vesicles indicate that it is possible to alter the lipid content of cultured mammalian cells after short incubations with liposomes of defined content^{6,7}. We report here that brief treatment with liposomes containing ergosterol can sensitise L1210 murine leukaemia cells to the subsequent action of amphotericin B.

Liposomes containing sterol were prepared using methods described by Weissmann⁸ and Cooper⁹. Egg yolk lecithin (EYL, Sigma) 40 mg alone or EYL 40 mg and ergosterol (Sigma) 80 mg, were dissolved in chloroform to assure complete mixing. The mixture of sterol and phospholipid was taken to dryness under nitrogen and 10 ml of buffer (100 mM NaCl, 15 mM KCl, 5 mM TES (N-Tris[hydroxymethyl]methyl-2-aminoethane sulphonic acid), 10 mM dextrose, 0.1 mM EDTA; pH 7.4) were added. This mixture was sonicated in a Branson Sonifier (Heat Systems Inc.), at an output setting of 60 W in a nitrogen atmosphere for 1 h at 3 °C. After sonication, the suspension was centrifuged at 30,000g for 10 min to remove non-dispersed lipids. Final phospholipid concentration was measured by the method of Bartlett¹⁰. Final ergosterol concentration was determined by the cholesterol method of Rudel and Morris¹¹, using ergosterol for the standard curve. Before incubation with cells, liposomes were diluted with buffer and medium to yield a final phospholipid concentration of 0.4 mg ml⁻¹ and a final ergosterol concentration of 0.05 mg ml⁻¹. Thin layer chromatography of the liposome preparation revealed the expected amount of phospholipid and sterol with less than 2% lysophosphatidylcholine contamination.

L1210 mouse leukaemia cells which had been in stationary phase for 5 h were adjusted to 7 × 10⁵ cells per ml with fresh medium. Aliquots of this suspension were placed in 75-cm² Falcon flasks, to which (1) buffer without liposomes, (2) EYL liposomes in buffer, or (3) EYL liposomes containing ergosterol in buffer, were added to yield a final cell concentration of 5.5 × 10⁵ per ml. Cells were incubated for 1 h at 37 °C, collected by centrifugation at 300 g for 5 min, and washed twice with fresh medium. They were resuspended at 1 × 10⁵ cells per ml in fresh medium (10 ml) containing amphotericin B or the solvent, DMSO, in 25 cm² Corning flasks. Cells were incubated for 2 h at 37 °C, washed twice more and resuspended in fresh medium.

After treatment and washing, one or more of the following techniques were used to assess cell survival. (1) Cell number was determined immediately after incubation in medium containing amphotericin B. (2) Trypan blue exclusion—washed cells in 0.5 ml medium were mixed with 0.1 ml 0.4% trypan blue in normal saline—after 5 min, 200 cells were counted and the number of cells excluding stain determined. (3) Cell number was determined after resuspension in fresh medium and 45 h additional incubation at 37 °C.

L1210 cells which were first incubated for 1 h with EYL liposomes containing ergosterol, and then treated for 2 h with amphotericin B at concentrations of 4-8 µg ml⁻¹ were reduced in number when counted immediately after the incubation period with amphotericin B (Fig. 1). These cell populations were unable to exclude Trypan blue effectively (Table 1), and phase microscopic examination revealed many cells undergoing lysis and much cellular debris. Treatment of cells with buffer alone or EYL liposomes without ergosterol did not confer such sensitivity to the action of amphotericin B.

Cells first treated with EYL liposomes containing ergosterol, exposed to amphotericin B, then incubated without ergosterol or amphotericin B for a further 45 h, exhibited growth inhibition and/or continuing diminution of cell number (Fig. 1). This effect was dose dependent over an amphotericin B concentration range of 2-8 µg ml⁻¹. Liposome treatment without subsequent amphotericin B

exposure was without effect. Amphotericin B treatment of cell populations previously incubated with buffer or EYL liposomes did not result in such reduction in cell number after 45 h further incubation (Fig. 1).

All cell populations examined immediately after the 1-h incubation period with buffer or liposome preparations excluded Trypan blue effectively. There was no change in cell number when these populations were compared with untreated cells. No cells were found adherent to vessel surfaces.

We have demonstrated that brief exposure to liposomes containing ergosterol can confer amphotericin sensitivity to one type of murine leukaemia cell. Other workers have demonstrated that *Tetrahymena pyriformis* cells grown for 36–40 h in culture medium containing ergosterol will incorporate ergosterol into their cell membranes and then become sensitive to the action of amphotericin B¹. In an earlier communication¹² it was reported that 22 h of growth in culture medium containing ergosterol was required for L1210 cells to become markedly affected by amphotericin B.

The mechanisms of ergosterol and amphotericin B action are not fully understood. Ergosterol may nonspecifically prepare the cell membrane for optimum interaction with amphotericin B by making the membrane more rigid or ordered¹³. Alternatively ergosterol could uniquely combine with amphotericin B and change membrane configuration so that membrane disruption occurs and cell lysis ensues^{14,15}.

Liposomes have been shown to interact with a variety of mammalian cell types^{7,8}. Although the precise nature of the interaction is not clear, the liposomes can transfer large amounts of lipids to cells and alter cellular lipid content⁶. Since sensitisation to amphotericin B might require the presence of a specific sterol at the membrane level, we presume that sensitisation observed after treat-

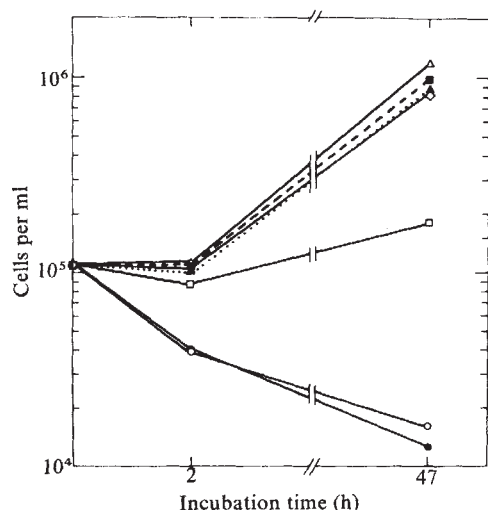


Fig. 1 Time course of effect of amphotericin B on cell number. L1210 cells (from Dr V. Bono Jr, NCI) were incubated for 1 h in the buffer, EYL liposomes or EYL liposomes containing ergosterol. Cells were washed, treated for 2 h with amphotericin B or dimethylsulphoxide (DMSO), then washed again. One aliquot of cells was counted and another was resuspended in fresh medium and incubated for a further 45 h and then counted. Cells treated with EYL liposomes containing ergosterol followed by treatment with DMSO (Δ), or amphotericin B at $2 \mu\text{g ml}^{-1}$ ($\diamond$), $4 \mu\text{g ml}^{-1}$ ($\square$), $6 \mu\text{g ml}^{-1}$ ($\circ$) or $8 \mu\text{g ml}^{-1}$ ($\bullet$). Cells treated with EYL liposomes then amphotericin B $6 \mu\text{g ml}^{-1}$ ($\blacksquare$); cells treated with buffer (without any liposomes) then amphotericin B $6 \mu\text{g ml}^{-1}$ ($\blacktriangle$). RPMI 1630 medium was from NIH Media Unit. In all experiments media were supplemented with 20% foetal calf serum (Flow) and gentamicin $40 \mu\text{g ml}^{-1}$ (Schering). Foetal calf serum was heat inactivated at 56°C for 30 min. Cell number was estimated with a Coulter Counter. Amphotericin B (Calbiochem) was freshly prepared in DMSO to give a 20 mg ml^{-1} stock solution. The DMSO concentration in incubation mixtures did not exceed 0.1% (v/v).

Table 1 Effect of treatment with liposomes containing ergosterol on Trypan blue exclusion

Amphotericin B ($\mu\text{g ml}^{-1}$)	Cells excluding Trypan blue (% of buffer control)	
	EYL liposomes containing ergosterol	EYL liposomes alone
0	94	94
2	73	85
4	44	78
6	15	85

L1210 cells were incubated for 1 h with buffer, EYL liposomes or EYL liposomes containing ergosterol. Cells were washed, treated for 2 h with amphotericin B, washed again, and resuspended in fresh medium. 0.5 ml of cells in fresh medium was mixed with 0.1 ml 0.4% Trypan blue in normal saline. After 5 min, 200 cells were counted and number excluding trypan blue determined.

ment with liposomes containing ergosterol was due to addition of this specific sterol to the membrane region.

As drug carriers, liposomes offer several advantages over conventional modes of drug administration. It has been suggested that they can transport high concentrations of otherwise toxic agents and perhaps can be directed to selected tissue targets^{16–19}. Our data indicate that in addition, specifically constituted liposomes may sensitise certain cell types to chemotherapeutic agents to which they were previously resistant. This study demonstrates a novel use of liposomes for the purpose of functionally altering the membranes of mammalian cells.

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FRED J. SCHIFFMAN

Laboratory of Medicinal Chemistry
and Biology,

IRWIN KLEIN

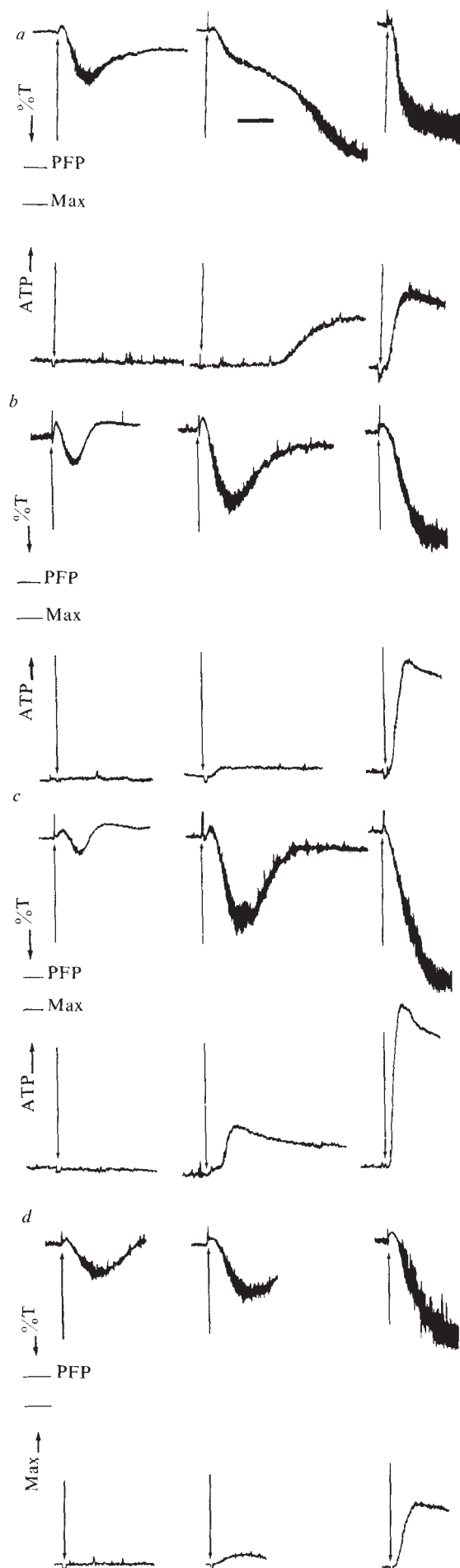
Laboratory of Molecular Biology,
National Cancer Institute,
Bethesda, Maryland 20014

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Prostaglandin endoperoxides and thromboxane A₂ can induce platelet aggregation in the absence of secretion

PLATELET aggregation and secretion are widely studied not only because they are believed to reflect the major *in vivo* platelet function, formation of the primary haemostatic plug, but also because they involve fundamental cellular regulatory mechanisms. The synthesis of biologically active prostaglandin (PG) intermediates (endoperoxides and thromboxanes) seems to be an important step in the



regulation of platelet function^{1,2}, but the physiological roles and mechanism(s) of these compounds are unknown. It is claimed by Malmsten *et al.*³ and Samuelsson *et al.*⁴ that PG endoperoxides and thromboxane A₂ cause platelet aggregation only by inducing platelets to secrete ADP that in turn causes aggregation. In view of observations that platelets deficient in releasable ADP (storage pool-deficient platelets or platelets depleted of storage granules) still aggregate in response to arachidonic acid⁵⁻⁷, the precursor of the intermediates, the validity of this claim has been open to question. We have investigated platelet stimulation by PG endoperoxides (PGG₂ and PGH₂), an endoperoxide analogue ((15S)-hydroxy-11 α , 9 α -(epoxy-methano) prosta-5Z, 13E-dienoic acid; U-46619), thromboxane A₂-like material and arachidonic acid, using a new instrument that simultaneously monitors aggregation and secretion in the same platelet suspension⁸, permitting careful analysis of the relationships between aggregation and secretion. We report here that although high concentrations of the endoperoxide analogue, the natural endoperoxides and thromboxane A₂ induce both platelet aggregation and secretion, low concentrations cause aggregation without detectable secretion.

Secretion was monitored by recording luminescence resulting from the interaction of released ATP (secreted simultaneously with ADP) with firefly luciferase and

Fig. 1 Simultaneous recording of aggregation and secretion induced by (a) PG endoperoxide analogue (U-46619), (b and c) natural endoperoxides (PGH₂ and PGG₂) and (d) thromboxane A₂ added to stirred platelet-rich plasma. These experiments represent typical results obtained with low, intermediate and saturating (maximal aggregation and secretion of ATP) concentrations of each stimulus. Secretion of ATP (lower traces) was measured by the firefly luminescence assay, with the gain of the instrument set so that maximum release with a high concentration of thrombin gave half-chart deflection (max), which corresponds to about 5 μ M ATP or 1 nmol per 10⁸ platelets. In the three experiments where the lowest concentrations of the analogue, PGH₂ or PGG₂ were added, the gain was doubled to detect even trace secretion of ATP. The sensitivity of the measurement in these conditions is such that a pen deflection of twice the noise amplitude is caused by less than 50 nM ATP. Since others^{19,10} have shown that ATP and ADP are secreted in parallel with the ATP/ADP ratio between 0.5 and 1.0, we would detect secretion of 0.1 μ M ADP. The decrease in the signal seen at the end of some curves does not represent uptake or metabolism of ATP by platelets, but is due to the well-known property of the assay system to show a decay in luminescence^{21,22}. Aggregation (upper traces) was measured simultaneously in the same cuvette, with the limit set with platelet-free plasma (PFP) as indicated. Reactions were initiated by addition of the stimulating agents to stirred platelet-rich plasma, except for experiments with PGH₂ and PGG₂, where the reaction was initiated by addition of platelet-rich plasma to a cuvette to which the endoperoxide had previously been added and the solvent (acetone) evaporated with a gentle stream of nitrogen. Thromboxane A₂-like material was generated by incubation of dog platelet-rich plasma with 0.5 mM arachidonic acid, as described by Chignard and Vargaftig¹². Aliquots of the dog platelet-rich plasma containing thromboxane A₂-like material were transferred directly to human platelet-rich plasma that had been pre-incubated with indomethacin (15 μ M for 30 s) to prevent stimulation by arachidonic acid. The concentration of thromboxane A₂-like material was varied by transferring different volumes or by varying the time of incubation with arachidonic acid. Control dog platelet-rich plasma had no effect on human platelets, and we confirmed the observations of Chignard and Vargaftig¹² that dog platelets neither aggregate nor secrete in response to arachidonic acid, although they do metabolise arachidonic acid to PGs and thromboxanes. All experiments were carried out with the cell compartment at 37°C, and the chart speed was 60 s inch⁻¹ (time bar in top trace). Each experiment was carried out on platelet-rich plasma (counts not standardised) from at least three donors. Although there were some quantitative differences between donors, the pattern of responses was consistent and the data shown are from a single donor, so that comparisons between stimuli are valid. The concentrations of stimuli (for low, intermediate and high concentrations) were: endoperoxide analogue 0.5, 1.2 and 2.2 μ M; PGH₂ 0.5, 0.8 and 9.4 μ M; PGG₂ 0.3, 0.5 and 2.8 μ M. Exact concentrations of thromboxane A₂-like material were not determined.

luciferin as described previously^{9,10}. The reaction was carried out in a new cell compartment designed to permit simultaneous measurement of platelet aggregation and secretion. Details of this technique have been described previously⁵. PGG₂ and PGH₂ were prepared essentially as described elsewhere¹¹. Final concentrations quoted are based on the specific activity of the radioactive arachidonic acid used in their biosynthesis. Thromboxane A₂-like material was generated by incubation of dog platelet-rich plasma with arachidonic acid¹². Although dog platelets do not aggregate or secrete nucleotides or other contents of dense bodies in response to arachidonic acid, they do synthesise PG endoperoxides and thromboxane A₂, which will aggregate human platelets. Since Smith *et al.*¹³ have shown that in the conditions used (incubation in plasma at 37 °C), thromboxane A₂ activity persists after complete disappearance of the endoperoxides, we refer to the active component as thromboxane A₂. Human platelet-rich plasma, prepared from whole blood collected in 0.1 volume 3.8% sodium citrate¹⁴, was kept at room temperature and was used within 3 h. Experiments were at 37 °C. All donors denied taking any drug for at least 10 d before venipuncture.

The effects of the unstable PG intermediates and the stable endoperoxide analogue on platelets were observed by recording the precise temporal relationships of aggregation and secretion (Fig. 1). Each compound was tested over a range of concentrations from an amount just sufficient to cause an observed effect to an amount greater than that required for the maximal effect. Individual donors varied in their responsiveness to the endoperoxides, but PGG₂ was consistently more potent than PGH₂. Examples of typical results for low, intermediate and saturating concentrations of each compound are shown in Fig. 1.

At low concentrations, each compound induced aggregation in the complete absence of detectable secretion (that is, primary aggregation), directly refuting the claim^{3,4} that the endoperoxides induce platelet aggregation only through release of ADP.

At intermediate concentrations, some difference was observed between the stable endoperoxide analogue and the unstable PG intermediates. The analogue induced biphasic aggregation, with secretion beginning only after a long lag and in parallel with the second wave of aggregation. This is characteristic of the response of platelets to other primary aggregating agents such as ADP and adrenaline^{14,15}. In contrast, when the stimulating agent was PGH₂ or PGG₂ we never observed biphasic aggregation or a long lag before secretion; instead intermediate concentrations of these compounds have consistently induced completely reversible aggregation accompanied by secretion. (Such readily reversible aggregation in platelets undergoing secretion is unusual.) This difference indicates that the synthetic endoperoxide analogue may not exactly mimic the platelet stimulating properties of the natural endoperoxides. Thromboxane A₂-like material induced aggregation and secretion with a temporal relationship similar to that for the naturally occurring endoperoxides.

Addition of increasingly higher concentrations of each stimulus resulted in more rapid and extensive aggregation-secretion curves until a maximal response was attained. In the case of maximal response, aggregation and secretion proceeded as temporally parallel events. With each stimulus, the secretion curves resulting from high concentrations of PGG₂ were nearly identical to those obtained previously with other potent stimuli (for example, thrombin or A23187) (ref. 15)), with no evidence of the extremely fast secretion reported by Malmsten¹⁶ and Corey *et al.*¹⁷, who observed complete release within 5 s.

Arachidonic acid also induced aggregation and secretion, probably because it is the substrate for PG and thromboxane synthesis¹⁸. Arachidonic acid differed from the other compounds tested in that aggregation was always

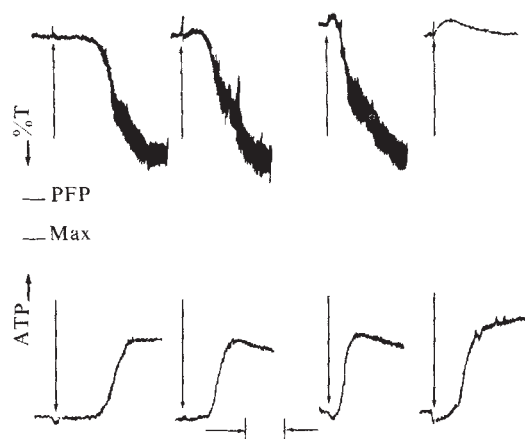


Fig. 2 Simultaneous recording of aggregation and secretion induced by arachidonic acid. The experiments were as described in Fig. 1. The fourth trace was done in the absence of stirring. Note that the higher concentrations of arachidonic acid lead to progressively shorter lag times and more rapid kinetics of secretion, but the total ATP secreted does not increase significantly. Lower concentrations of arachidonic acid also induced aggregation and secretion in parallel, with increasingly longer lags, until a threshold concentration was reached below which there was neither aggregation nor secretion (approximately 100 μ M in the experiment shown). Sodium arachidonate concentrations (from left to right): 110, 190, 640, 640 μ M.

accompanied by secretion (Fig. 2). (This is not inconsistent with a report³ that arachidonic acid causes aggregation of platelets that have been depleted of their secretory granules by treatment with thrombin, but suggests that aggregation and secretion may be independent, parallel responses of platelet activation¹⁵.) This difference in activity of the PG intermediates and arachidonic acid suggests that there may be a difference between the effects of extracellular intermediates and the same compounds generated intracellularly. That is, primary aggregation may be due to interactions of the intermediates with the outer membrane, whereas activation by arachidonic acid may involve an intracellular action of the same intermediates.

We also observed that at higher concentrations, each stimulus, as shown in Fig. 2 for arachidonic acid, is able to induce secretion in the virtual absence of aggregation (unstirred suspension), indicating that secretion is not dependent on aggregation, as shown for other stimuli, such as thrombin¹⁵.

We conclude that endoperoxides and thromboxane A₂ can induce aggregation without secretion of nucleotides, that higher concentrations of endoperoxides and thromboxanes can induce secretion independent of aggregation, and that human platelet aggregation can spontaneously reverse even when accompanied by secretion. This is the first direct evidence that PG intermediates can cause primary aggregation of normal human platelets.

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ISRAEL F. CHARO
RICHARD D. FEINMAN
THOMAS C. DETWILER

Department of Biochemistry,
SUNY Downstate Medical Center,
Brooklyn, New York 11203

J. BRYAN SMITH
CAROL M. INGERMAN
MELVIN J. SILVER

Cardeza Foundation,
Thomas Jefferson University,
Philadelphia, Pennsylvania 19107

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New polymorphism of platelet membrane glycoproteins

THE surface of the human blood platelet is rich in carbohydrate and at least four glycoprotein components have been resolved by gel electrophoresis of isolated platelet plasma membranes¹. Two platelet disease states are characterised by a diminution or absence of certain of these glycoproteins²⁻⁵: in the case of Bernard-Soulier disease, glycoprotein I (molecular weight (MW) 150,000) is lacking while platelet membranes isolated from patients suffering from Glanzmann's thrombasthenia lack glycoprotein II (MW 120,000). While screening several patients with ill-defined but clinically evident haemostatic problems, we have now identified two unrelated individuals who show an abnormal polymorphic pattern of glycoprotein distribution within their platelet membranes and a concomitant reduction in the ability of the intact platelets to bind thrombin.

Polyacrylamide gel electrophoresis of normal platelet plasma membranes gives a pattern of four major bands which stain with the periodate-Schiff reagent¹ (Fig. 1a). These bands have been designated glycoproteins I, II, III and IV and have electrophoretic mobilities corresponding to molecular weights of 150,000, 120,000, 98,000 and 87,000, respectively. These four peaks are in the ratio of 55:28:6:11, averaged from 37 samples, and show no age or sex-related differences.

In the case of platelets from two individuals identified

Fig. 1 Glycoprotein patterns from normal (a) and abnormal (b) platelets. A platelet membrane fraction was subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate and β -mercaptoethanol followed by staining with periodate-Schiff reagent, as previously described¹.

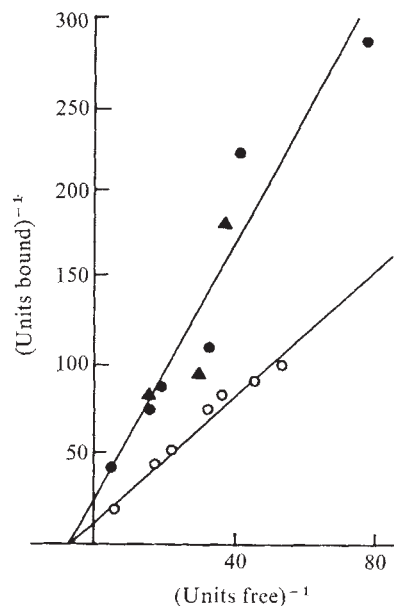
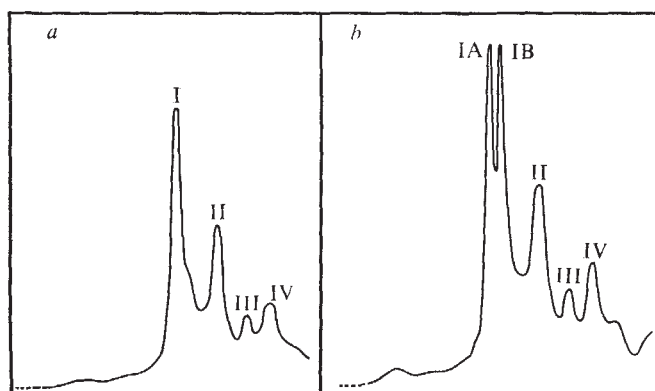


Fig. 2 Thrombin binding to normal and abnormal platelets. Control, $\circ$: patient 1, $\blacktriangle$: patient 2, $\bullet$. Binding studies were performed by the Majerus technique using cacodylate buffer¹² and highly purified human thrombin¹⁴ (2,850 NIH units mg^{-1}) (ref. 14) iodinated with ^{125}I and were corrected for nonspecific binding.

during these experiments we found a pattern which differed from normal in that there was a double band in the region of glycoprotein I (Fig. 1). The first band corresponded in electrophoretic mobility to glycoprotein I while the mobility of the second band indicated a molecular weight 10,000–12,000 lower. Each of these components was present in about one-half the amount of glycoprotein I in normal platelets. Combining the values for peaks IA and IB gives ratios (IA+B:II:III:IV) of 63:23:7:7 and 63:18:9:10, respectively, for the two patients which are ratios similar to those found in normal controls.

Several different types of experiment were carried out to determine whether this polymorphism was an intrinsic property of the membrane glycoproteins in these two cases or could have arisen from extraneous causes. Since glycoprotein I is known to be particularly sensitive to proteolytic digestion^{1,3} the possibility of increased proteolytic activity in the patients' serum was investigated by incubating normal platelets with samples of plasma from the two subjects for 8 h at 37 °C; this did not result in the appearance of a double band in the membranes subsequently isolated from the treated platelets indicating that the double band did not result from extraneous proteolysis, nor was there any appearance of the second band on prolonged incubation of the platelets themselves indicating that it was not due to protease activity in the abnormal platelets. Peaks IA and IB were equally susceptible to added trypsin in intact platelets and a single PAS-positive band was observed in the soluble supernatant with an electrophoretic mobility indistinguishable from that of the macroglycopeptide obtained from normal platelets⁶. These observations suggested that differences in the electrophoretic mobility of peaks IA and IB probably were not due to structural differences in the macroglycopeptide portion of the two molecules.

Glycoprotein I seems to exist both in membrane-bound forms and in a form, glycocalicin, which is loosely bound within the platelet glycocalyx and is obtained in soluble form following platelet homogenisation¹. The relationship between glycoprotein I and glycocalicin is not clear, however, and they differ in their pattern of labelling with surface probes¹. Glycocalicin has been isolated and characterised⁷ and has been shown to be an inhibitor of

Table 1 Clinical laboratory studies

	Normal values	Subject 1 (female, aged 22 yr)	Subject 2 (female, aged 28 yr)
Platelet count (mm^{-3})	150,000–450,000	400,000	290,000
Bleeding time (min)	4–10	6	5
Platelet retention (%)	> 35	23	10
Prothrombin time (s)	12–14	13.2	13.0
Activated PTT (s)	< 35	32	35
Aggregation (%)			
Collagen (2 μg)	> 65	35	75
Adrenaline (2.5 μM)	> 70	6	85
ADP (10 μM)	> 70	39	90
Ristocetin (1.5 mg ml^{-1})	> 80	100	88
Thrombin (0.2 units ml^{-1})	> 65	74	84
VIII _{VWF} (%)	55–120	86	81
VIII _{AHF} (%)	50–200	100	115
PF-3 (s)	60	72	ND

Platelets were counted by phase microscopy. Platelet adhesiveness was measured by a modified Salzman technique¹⁶, bleeding times by the template technique of Mielke¹⁷ and prothrombin times, activated partial thromboplastin times (PTT) and one stage Factor VIII bioassay by standard methods. VIII_{VWF} assays were performed by the quantitative technique of Weiss¹⁸, platelet factor 3 (PF 3) availability was performed by the kaolin technique¹⁹ and platelet aggregation by the turbidimetric method of Born²⁰. ND, Not determined.

thrombin-induced platelet aggregation⁸ and to have thrombin-binding activity associated with the (nonglyco) peptide 'tail' portion of the molecule (T.O. and G.A.J., submitted for publication). Although there was no resolution into two electrophoretic peaks, glycoprotein present in the soluble portion of the platelet homogenate in these two cases showed a broader distribution of electrophoretic mobilities as compared with controls and the amount present was in the low-normal range.

Quantitative studies of thrombin-binding were carried out by the filtration technique of Majerus⁹ in the high affinity range (0.020–0.080 units) considered to be significant for thrombin action under physiological conditions^{9–11}. Binding studies were carried out in cacodylate buffer which gives a 10-fold increase in binding efficiency¹². The number of thrombin-binding sites per platelet were calculated from double reciprocal plots according to Steck and Wallach¹³ using computer-assisted analysis to obtain best fit (Fig. 2). Data calculated in this way gave a value of 5,400 thrombin binding sites per platelet for normal controls but only one half that, 2,400 per platelet, for the two patients showing the glycoprotein abnormality. The reciprocal plots intersected at the abscissa indicating that there is no difference in the affinity for thrombin between the patients and controls: a value of 0.35 units ml^{-1} was determined for half saturation of thrombin binding sites.

Although both the glycoprotein polymorphism and the decrease in thrombin binding were found consistently in these patients it was not possible to correlate these biochemical observations with clinical laboratory data (Table 1). In one case (subject 2) all values were within the normal range whereas in the other case (subject 1) there seemed to be an associated release defect. Associated release defects have also been noted in other cases of abnormal haemostasis¹⁵. It may be noted that thrombin-induced aggregation was normal in both patients. Binding of thrombin to platelets is kinetically complex^{9–11}, however, and it is not possible to predict a relationship between the number of thrombin receptors present and the extent of aggregation to be expected.

No evidence for a genetic basis for the polymorphism could be obtained within the limited family groups available: subject 1 gave no history of family bleeding problems while subject 2 gave a family history of easy bruising in her father, sister and daughter but without the more marked bleeding episodes of her late mother and herself. Examination of the three living family members failed to reveal any glycoprotein abnormality and all gave normal values for VIII_{AHF}, VIII_{VWF}, in routine coagulation and aggregation studies, and in thrombin binding.

Although it is not known whether this is the primary cause of the bleeding defects observed in these two individuals, the two cases described here show identical patterns of glycoprotein polymorphism and decreased thrombin binding capacity. Since the macroglycopeptide portions of the two components seem to be identical on the basis of electrophoretic mobility, and since the thrombin-binding site of glycoprotein I/glycoprotein IIb is present in the peptide 'tail' (MW 45,000) of the molecule, these suggest that the lower molecular weight of the abnormal form of glycoprotein I may result from a deletion of structures associated with thrombin binding in that region. Direct correlation of the electrophoretic and functional data will require isolation and comparison of the two forms of glycoprotein I manifest in these individuals.

R. B. BOLIN
T. OKUMURA
G. A. JAMIESON

American National Red Cross,
Blood Research Laboratory,
Bethesda, Maryland 20014

Received 28 March; accepted 12 July 1977.

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Interaction of colchicine with phosphatidylcholine membranes

EXPERIMENTAL results have suggested that the alkaloid drug colchicine affects the lateral mobility of membrane components^{1–5}. To explain these observations, Wunderlich *et al.*⁶ have proposed two alternative hypotheses: the lipophilic

drug intercalates into the lipid bilayer in a manner analogous to cholesterol; or colchicine acts by binding to membrane protein. It is well documented that in lipid bilayers of both model and biological membranes, the methylene groups of the lipid fatty acyl chains are more mobile near the chain terminus than near the glycerol backbone of the phospholipid, resulting in a gradient of increasing fluidity in the non-polar phase of the bilayer interior⁷⁻¹². From nuclear magnetic resonance (NMR) and spin-label electron spin resonance (ESR) studies it has been found that cholesterol, by intercalating between the phospholipid fatty acyl chain, markedly increases the rigidity of the hydrocarbon phase of the bilayer¹³⁻¹⁹. We present here the results of a detailed spin-label study using phosphatidylcholine (PC) liposomes to show that, in contrast with cholesterol, colchicine does not detectably change the rigidity of PC bilayers.

The spin labels used (see Fig. 1), 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), 2-(3-carboxypropyl)-4,4 dimethyl-2-tridecyl-3-oxazolidinyloxy (C_5 nitroxide-labelled stearic acid), 2-(10-carboxydecyl)-2-hexyl-4,4-dimethyl-3-oxazolidinyloxy (C_{12} nitroxide-labelled stearic acid) and 2-(14-carboxytetradecyl)-2-ethyl-4,4-dimethyl-3-oxazolidinyloxy (C_{16} nitroxide-labelled stearic acid) are convenient probes for the measurement of the effects of a perturbant on the hydrophobic phase of a lipid bilayer. An increase in the rigidity of the matrix of the fatty acyl chains is reflected by an increase in the hyperfine splitting ($2A'_{zz}$) in the ESR spectrum of the stearic acid spin labels and by a decrease in the parameter f of the TEMPO ESR spectrum²⁰ (see Fig. 1).

The results of replicate experiments indicate that on increasing the concentration of colchicine there was no change in the stearic acid spin label ESR spectra and no change in the f -value of TEMPO (ref. 20). As an example, ESR spectral parameters for 0 and 30 mg ml⁻¹ colchicine are listed in Table 1. However, an increase in cholesterol to phospholipid molar ratios over the range of the colchicine to phospholipid molar ratios (0.0-1.0) used in this experiment greatly increases the rigidity of the fatty acid acyl chains¹³⁻¹⁹.

From these results, there is no evidence to suggest that colchicine, like cholesterol, decreases the fluidity of the lipid

Table 1 ESR data summary*

Label	Spectral parameter	
	Colchicine (0 mg ml ⁻¹)	Colchicine (30 mg ml ⁻¹)
TEMPO	$f = 0.38 \pm 0.01$	$f = 0.38 \pm 0.01$
C_5	$A'_{zz} = 26.0 \pm 0.2$ G	$A'_{zz} = 26.0 \pm 0.2$ G
C_{12}	Spectra superimposable; A'_{zz} is difficult to extract	A'_{zz} is difficult to extract
C_{16}	$A'_{zz} = 14.3 \pm 0.2$ G†	$A'_{zz} = 14.3 \pm 0.2$ G†

Phosphatidylcholine was extracted from egg yolks by the method of Singleton *et al.*²³ with the exception that the acetone, used for precipitation of the crude phosphatides, was used at dry ice temperature under N₂. This speeded precipitation and retarded atmospheric oxidation of the crude phosphatides, as determined by thin-layer chromatography. TEMPO was prepared by the method of Briere *et al.*²⁴. The nitroxide-labelled stearic acids were purchased from Syva. PC and the desired spin label in 1:200 molar ratio with PC were dissolved in N₂-saturated distilled chloroform and dried to a film using a rotary evaporator. The last traces of chloroform were removed under vacuum (about 6.0×10^{-5} atm) for 2 h. N₂-saturated buffer, 10 mM Tris, 5 mM KCl, pH 7.4, was added to the mixture to give a 100 mg ml⁻¹ PC dispersion. The slurry was agitated until no PC was seen adhering to the walls of the flask. Colchicine (Sigma), in buffer solution, was added to 0.5-ml aliquots of the PC dispersion to give a final PC concentration of 50 mg ml⁻¹ and colchicine concentrations ranging from 0 to 30 mg ml⁻¹. The PC-colchicine solutions were shaken on a vortex mixer for 1 min and allowed to equilibrate for at least 2 h at room temperature under N₂ before spectra were taken. The mixtures were vortexed again immediately before the taking of spectra. A parallel series of experiments was done with lipid dispersions sonicated as described by Godici and Landsberger for 25 min. Colchicine was added to these sonicated dispersions as described above. In one series of experiments, PC and colchicine in concentrations of 0 and 30 mg ml⁻¹ were co-sonicated in conditions previously described⁸. ESR spectra were obtained with Varian E-4 and E-12 spectrometers.

*Average values of replicate experiments using unsonicated dispersions.

† A'_{zz} is one-half the distance between the base-line crossings of the high and low magnetic field peaks of the ESR spectrum.

fatty acyl chains. The data also rule out any detergent-like interaction between colchicine and lipid membranes. Although the possibility is not rigorously precluded that colchicine inhibits lateral diffusion of lipids, as has been described for cholesterol^{21,22}, and hence that of membrane protein, this seems extremely unlikely. Colchicine, in concentrations similar to these cholesterol studies^{21,22} and up to 10⁴ times greater than those known to affect the lateral mobility of membrane components¹⁻⁵, does not affect the motional freedom of the lipid hydrocarbon chains.

Thus, we have tried to differentiate between the two hypotheses suggested by Wunderlich *et al.*⁶. We conclude that colchicine does not interact with the lipid bilayer in a manner analogous to that of cholesterol and that it seems that colchicine might interact with membrane and/or microtubular protein¹⁻⁶.

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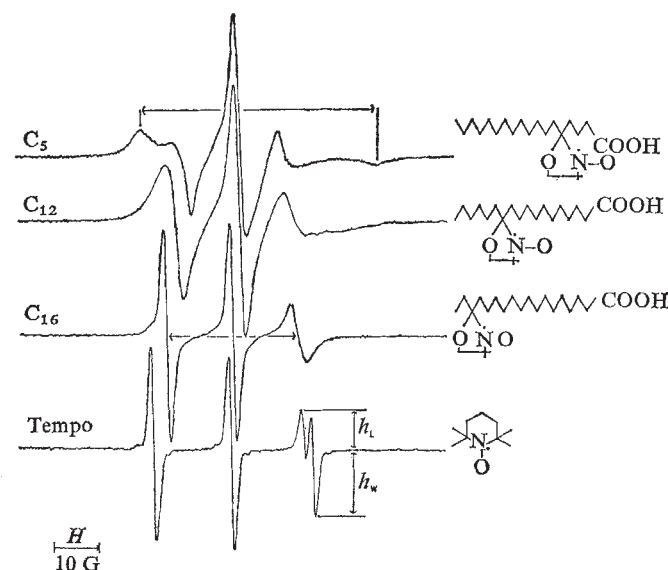
LAWRENCE D. ALTSTIEL
FRANK R. LANDSBERGER

The Rockefeller University,
New York, New York 10021

Received 31 May; accepted 11 July 1977.

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Fig. 1 ESR spectra from C_5 , C_{12} , C_{16} and TEMPO-labelled unsonicated lecithin dispersions containing 30 mg ml⁻¹ colchicine. For the C_5 and C_{16} spectra, $2A'_{zz}$ is defined by the horizontal distances indicated. From the TEMPO spectrum, the quantity f is defined as $h_L/(h_L + h_w)$, where h_L and h_w to a first approximation are proportional to the fraction of TEMPO in the lipid and water phases, respectively. H is the applied magnetic field.



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Are benzodiazepines GABA antagonists?

ALTHOUGH benzodiazepines are widely used in routine medical practice their mechanism of action remains controversial^{1,2}. Several authors have suggested that the action of benzodiazepines is to potentiate GABA-ergic processes in the central nervous system³⁻⁵. In addition, a number of studies have shown the effect of benzodiazepines to be antagonised by γ -aminobutyric acid (GABA) antagonists^{6,7}. But, Steiner and Felix⁸ and Gähwiler⁹ have adopted the opposite view and reported that in the cerebellum and Deiters nucleus benzodiazepines antagonise the inhibitory action of GABA. On the other hand, Dray and Straughan⁶ have shown microiontophoretic applications of benzodiazepines to be inhibitory on the spontaneous and glutamate activity of neurones of the brain stem and were unable to confirm that benzodiazepines antagonise the action of GABA. Curtis *et al.*¹⁰ were also unable to confirm the results of Steiner and Felix⁸ on the neurones of the cerebellum and spinal cord. In the recent paper by Squires and Braestrup¹¹ a direct interaction of benzodiazepines with GABA-receptors has been discarded. We have investigated the interaction between chlordiazepoxide (Librium) and GABA by observing the effects of simultaneous microiontophoretic application of both agents on the spontaneous and glutamate-evoked firing of single neurones of sensorimotor cortex of the rabbit and here we report that the actions of the two are synergistic.

The experiments were performed on seven adult rabbits anaesthetised with hexobarbitone sodium and artificially ventilated. Microiontophoresis and recording was from five to seven barrelled micropipettes of 4-6 μ m overall tip diameter. The central recording barrel was filled with 2M NaCl and only those neurones with extracellular action potentials of 1 mV or greater

Fig. 1 The dose-dependent action of microiontophoretically applied chlordiazepoxide hydrochloride (CDP) on the glutamate-evoked firing of a sensorimotor neurone. The iontophoretic application and the current passed in nA (1×10^{-9} A) are indicated by the horizontal bar and the figures above them. The rate meter was reset every 3 s. Retaining currents in all the experiments were between 15 and 45 nA.

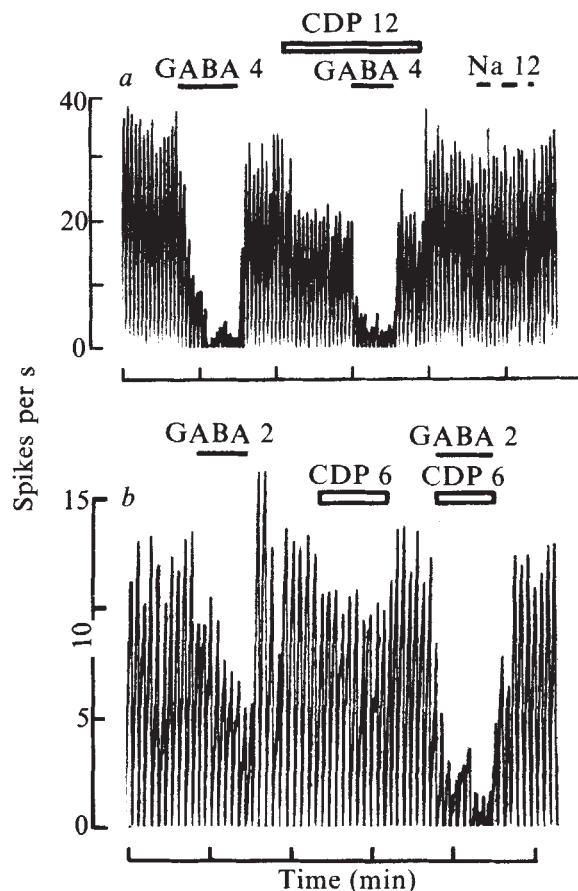
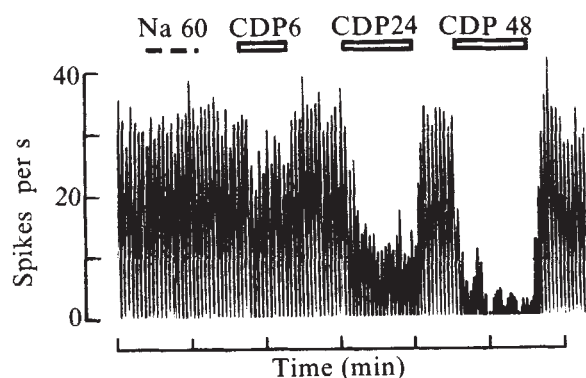


Fig. 2 Synergism between chlordiazepoxide and GABA applied microiontophoretically to the same neurone. *a*, The failure of CDP to antagonise the inhibitory action of GABA. *b*, The summation of submaximal inhibition evoked by GABA and CDP. In (*a*) the activity of the neurone was maintained by the application of glutamate (3 nA) and the rate meter was reset every 3 s in (*a*) and 5 s in (*b*).

were studied. The concentration of chlordiazepoxide was 0.2M ($pH = 3.0$), and the other barrels contained sodium glutamate (1 M; $pH = 7.5$), GABA (1 M; $pH = 3.0$) and NaCl (3 M). GABA was applied with the minimum microiontophoretic ejecting current required to induce a marked and stable inhibitory effect of the agent on the spontaneous or glutamate-evoked activity of a given neurone. The effects of current flow on neuronal activity was minimised using standard current balancing techniques.

Figure 1 shows the marked reduction in activity evoked by a microiontophoretic application of chlordiazepoxide to a neurone of the sensorimotor cortex. This inhibitory action of chlordiazepoxide increased with the applied current and this effect was found on all 22 cells studied; in no trial did chlordiazepoxide increase neuronal activity.

Figure 2*a* shows the failure of chlordiazepoxide to antagonise the action of GABA. Similar results were obtained on 14 neurones and no antagonism between chlordiazepoxide and GABA was observed during the application of chlordiazepoxide with currents between 2 and 120 nA. Similar results were obtained in two additional experiments in which anaesthesia was induced and maintained with urethane or chloralose.

In complete contradiction to the suggestion that chlordiazepoxide antagonises the action of GABA we have found the inhibitory action of chlordiazepoxide to be synergistic with that of GABA. In Fig. 2*b*, for instance, small applications of both GABA and chlordiazepoxide which evoked only small degrees of inhibition combine to induce almost complete inhibition. Since in previous studies we observed systematically administered diazepam to potentiate the action of GABA on the spontaneous

activity of neurones in the sensorimotor cortex¹², we suggest that the actions of benzodiazepines and GABA are synergistic.

S. N. KOZHECHKIN

R. U. OSTROVSKAYA

*Institute of Pharmacology,
Academy of Medical Sciences of the USSR,
Moscow, USSR*

Received 16 January; accepted 30 May 1977.

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Agonist-antagonist properties of *N*-allyl-[D-Ala]²-Met-enkephalin

RECEPTOR displacement of the opiate antagonist naloxone requires concentrations of morphine and other opiate alkaloids similar to those required to elicit analgesia^{1–3} or inhibit ileal contraction^{4–5}. A new class of opiates with structures based on the discovery of endogenous, morphine-like pentapeptide—enkephalin⁶—also displace opiate receptor binding with potencies paralleling *in vivo* activities^{7–10}. Intrinsic activity (agonist or antagonist quality), a different dimension of opiate alkaloid effects *in vivo*, is closely correlated with the *in vitro* effects of sodium ion in the incubation medium: while antagonist binding to opiate receptors labelled by radioactive naloxone is almost unaffected by sodium ion addition, opiate alkaloid agonists become 10–60-fold weaker and mixed agonist-antagonists have intermediate downward shifts in apparent affinity after sodium ion addition^{11,12}. We present here an analysis of the sodium sensitivities of the major opiate peptides which have all been identified as ‘agonists’ thus far. In addition, we describe the synthesis, behavioural and *in vitro* receptor analysis of *N*-allyl-[D-Ala]²-Met-enkephalin (Fig. 1), a novel peptide whose structure was designed in analogy¹² with that of the alkaloid opiate antagonists¹³ in order to obtain a peptide opiate antagonist, if possible.

The sodium ion dependence of the receptor interactions of morphine, *N*-allyl-[D-Ala]²-Met-enkephalin and three opiate peptides is shown in Fig. 2. Like the prototype opiate agonist morphine, [D-Ala]²-Met-enkephalinamide (an enzyme-resistant enkephalin)¹⁴ and the C fragment of β -lipotropin (61–91) (refs 15–17) show large ‘sodium shifts’ of 25 and 17 respectively. This is consistent with recent reports which indicate that these peptides are morphine-like agonists as analgesics^{9,14,18,19}, and in the guinea pig ileum^{10,16}. It should be noted that Bradbury *et al.*¹⁵ reported a ‘sodium shift’ for the C-fragment of only 1.3. In



Fig. 1 Structure of *N*-allyl-[D-Ala]²-Met-enkephalin. *N*-allyl-L-tyrosine (A) was synthesised from *N*,*O*-dibenzoyl-L-tyrosine methyl ester by reaction with sodium hydride and allyl bromide and purified by countercurrent distribution. Reaction of A with *tert*-butoxycarbonyl azide gave the *N*-protected derivative (B). D-Alanylglycyl-L-phenylalanyl-L-methionine (C) was prepared by stepwise condensation using *N*-hydroxysuccinimide esters. The crystalline tetra-peptide gave the expected amino acid analysis. B was converted to the *N*-hydroxysuccinimide ester and coupled to C. Following deprotection the homogeneity of *N*-allyl-[D-Ala]²-Met-enkephalin was ascertained by countercurrent distribution, the product showing this amino acid analysis (residues per mol): glycine, 1.00; alanine, 0.94; methionine, 1.00; *N*-allyl-tyrosine, 0.85 (C-value 0.09 of that of leucine; *N*-allyltyrosyl peptides are resistant to hydrolysis and A is subject to destruction); phenylalanine, 1.00.

the same study, the sodium sensitivity ratio of morphine was only 7 in contrast to the larger values of 38 obtained in these experiments and values of 33–40 consistently obtained in previous studies^{1,11,20}. The methodological difference which probably accounts for the discrepant values¹⁵ is the lack of incubation at 0 °C, since low temperature is essential for demonstrating sodium effects reproducibly²⁰.

The sodium shift value of Met-enkephalin⁹ suggests a predominantly agonist nature, but falls within the range of opiates which display partial antagonist activity in some test situations¹¹. A recent study²¹, indeed, suggests that Leu-enkephalin despite its three to fourfold reduced affinity for brain opiate receptors^{7,8}, maintains intraventricular self-administration behaviour more readily than Met-enkephalin because it is a ‘purer’ agonist. No antagonist activity is detectable for Met-enkephalin in the guinea pig ileum²², however.

In striking contrast to morphine and the opiate peptide agonists, *N*-allyl-[D-Ala]²-Met-enkephalin has a small (2.5) sodium response ratio which clearly suggests antagonist properties if sodium effects on peptides are as relevant to *in vivo* pharmacological activity as they are for opiate alkaloids. Intraventricular injection of the putative peptide antagonist, *N*-allyl-[D-Ala]²-Met-enkephalin, however, does not decrease the anti-nociceptive response obtained when [D-Ala]²-Met-enkephalin is injected alone (Table 1). In fact, the allyl peptide injected alone is a weak analgesic, suggesting the presence of weak agonist activity compatible with its rather low affinity for opiate receptors. The ‘narcotic antagonists’ (nalorphine, levallorphan and so on) also elicit analgesia in the tail-flick test and produce sodium ratios in the 1.5–3.0 range¹¹.

The various pharmacological assays for opiates are known to differentially emphasise their agonist or antagonist qualities. For example, chronic morphine administration causes a dramatic ‘supersensitivity’ to opiate antagonist activity so that much lower doses of antagonists are required to precipitate opiate withdrawal signs²⁴. We therefore examined the ability of *N*-allyl-[D-Ala]²-Met-enkephalin to produce withdrawal signs in

Table 1 Behavioural effects of *N*-allyl-[D-Ala]²-Met-enkephalin

Analgesic effects		Tail-flick latency
Drug		(s $\pm$ s.e.m.)
Vehicle		3.9 $\pm$ 0.16 (<i>n</i> = 7)
<i>N</i> -Allyl-[D-Ala] ²		7.4 $\pm$ 1.49 (<i>n</i> = 6)*
[D-Ala] ²		10.4 $\pm$ 1.23 (<i>n</i> = 7)*
[D-Ala] ² + <i>N</i> -allyl-[D-Ala] ²		13.5 $\pm$ 0.65 (<i>n</i> = 7)**
Withdrawal	Vehicle (<i>n</i> = 4)	<i>N</i> -Allyl-[D-Ala] ² (<i>n</i> = 7)
Wet-dog shakes	0.75 $\pm$ 0.75	3.86 $\pm$ 2.0
Chewing	0	1.71 $\pm$ 0.36

To test analgesic effects 27 rats were implanted with 22 ga cannulae guides, the tips of which were aimed for an area 2 mm above the third ventricle (AP 3.0, LAT 0.0, DV 2.5)²³. Two weeks after the operation the animals were divided into four groups and injected in the third ventricle with either 7 μ l water (vehicle), 35 μ g *N*-allyl-[D-Ala]² in 7 μ l water, 20 μ g D-Ala in 4 μ l water or 20 μ g D-Ala followed immediately by 35 μ g *N*-allyl-[D-Ala]². Analgesic effects were assessed 15 min after injection with the tail-flick task as previously described¹⁴ with the exception that the tail-flick response limit was increased to 15 s. In withdrawal tests 10 rats were implanted with intracerebral cannulae guides as above. One week later all animals were implanted subcutaneously with one 75 μ g morphine pellet. Forty-eight hours later they were implanted with two additional pellets and 48 h after the second pellet implantation the animals were divided into two groups and injected with either 35 μ g *N*-allyl-[D-Ala]² or 7 μ l water. Total number of wet-dog shakes and jumps were recorded for 30 min after injection. Ptosis, chewing, teeth chattering, eye twitching, or squeal on touch²⁵ were rated at 10-min intervals during the same observation period. Only wet-dog shakes and chewing were found to increase following *N*-allyl-[D-Ala]².

**P* < 0.05 for comparison of drug effects with vehicle control.

†*P* < 0.05 for comparison of D-Ala + *N*-allyl-[D-Ala]² with D-Ala alone.

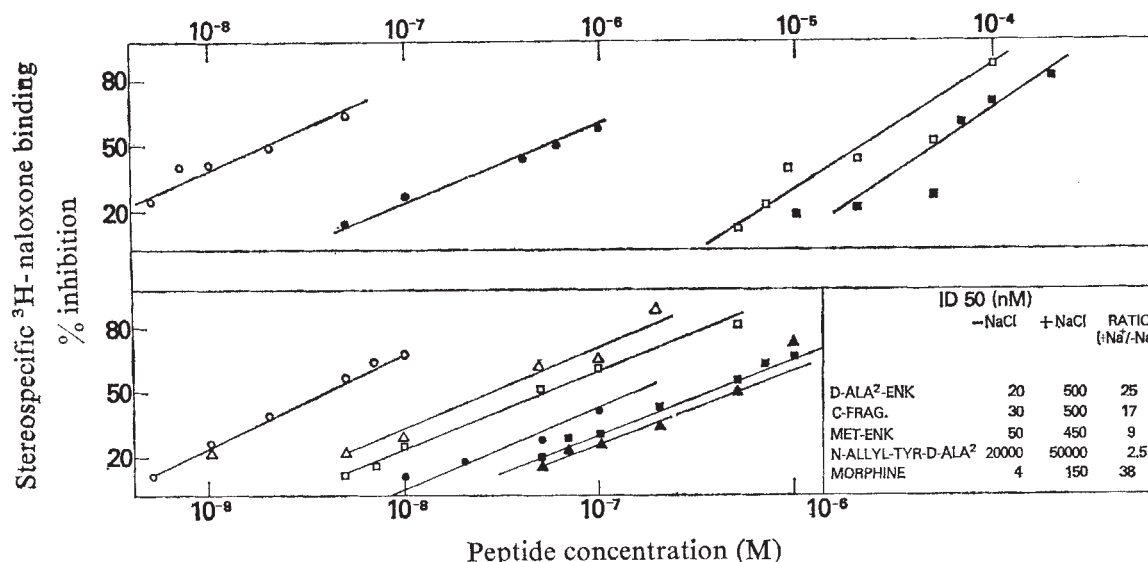


Fig. 2 Sodium sensitivity of the displacement of stereospecific ^3H -naloxone binding by several opiate peptides and morphine. Male Sprague-Dawley rats were killed by guillotine, their brain minus the cerebellum was rapidly removed and placed in 100 volumes of cold (4°C) 0.05 M Tris buffer (Trizma Preset pH crystals, pH 7.4 at 0°C) and homogenised with a Polytron set at number five for 15 s. The crude homogenate was centrifuged at $12,000g$ for 15 min, the supernatant discarded and the pellet reconstituted in 100 volumes of buffer. After repeating centrifugation and decanting, the pellet was resuspended in 10 ml of cold buffer by vortexing for 10 s and brief Polytron treatment. Membranes were prepared for use no later than 1 h after the final resuspension. The assay was incubated in duplicate in an ice water bath for 60 min in $10 \times 75\text{-mm}$ glass test tubes containing brain membranes (100 μl), peptides (abscissa), 50 μl ^3H -naloxone (2.4 nM, 20,000 c.p.m., $19.9 \text{ Ci mmol}^{-1}$), and 50 μl of 1 M sodium chloride (closed symbols) or buffer (open symbols), and sufficient Tris buffer required for a final volume of 500 μl . Membranes were added last. After 60 min each reaction mixture was briefly vortexed, then filtered under reduced pressure through Whatman GF/B filters and washed twice with 7 ml of ice-cold buffer in a filtration cycle of less than 10 s. Filters were transferred to vials and, after the addition of scintillation fluid (Aqualon, NEN) were counted in a Beckman LS-230 Scintillation counter at 45% efficiency. Control values of stereospecific ^3H -naloxone binding were calculated by subtracting nonspecific binding (c.p.m.) which occurred in the presence of 100 nM levallorphan. Control values from five experimental runs for total receptor binding and levallorphan binding were $1,625 \pm 72$ c.p.m. and 726 ± 172 c.p.m. respectively in the absence of sodium and $2,472 \pm 280$ c.p.m. and 429 ± 72 c.p.m. in the presence of sodium. ID_{50} and sodium ratio values, which varied less than 2-fold, were determined as the means from two experimental runs. All inhibition values between 15% and 85% for one experimental run are shown. In a: $\bullet$, $\circ$, [D-Ala]²-enkephalin; $\square$, $\blacksquare$, N-allyl-Tyr-D-Ala²-enkephalin. In b: $\bullet$, $\circ$, morphine; $\blacktriangle$, $\triangle$, C fragment (LPM 61-91); $\blacksquare$, $\square$, Met-enkephalin.

rats made dependent on morphine²⁵ in an attempt to demonstrate antagonist properties. Intraventricularly-injected morphine pellet-implanted rats indeed developed weak opiate withdrawal signs of 'wet-dog shakes' and chewing behaviour which were not present in the vehicle-injected controls (Table 1).

While further characterisation of the pharmacology of additional opiate peptides is required before a definite conclusion can be reached, these experiments show that the *in vitro* sodium effect provides information about the agonist-antagonist quality of opiate peptides when assessed in the very specific experimental conditions described here. The sodium effect probably occurs because receptors for endogenous as well as exogenous opiates exist as an inter-converting equilibrium between an agonist conformation and an antagonist conformation associated with a sodium binding site^{1,11,26,27}.

N-Allyl-[D-Ala]²-Met-enkephalin seems to be a weak agonist with mild antagonist character *in vivo* with a small sodium shift *in vitro* like that of opiate alkaloid partial antagonists. The clinically undesirable effects of chronic opiate administration have previously been demonstrated for [D-Ala]²-Met-enkephalinamide⁹, β -LPH (61-91) (ref. 28), and Met-enkephalin²⁸. Opiate peptides with partial antagonist quality might have diminished physical dependence and tolerance liability like the analgesics in the mixed agonist-antagonist benzomorphan group of opiates²⁹. In any case, these results suggest the feasibility of developing a 'pure' opiate peptide antagonist by *in vitro* analysis. Such an antagonist might more readily block the physiological actions of endogenous opiate peptide ligands; Lord *et al.* have already shown that the currently available alkaloid antagonists are surprisingly weak at blocking some actions of opiate peptide agonists³⁰.

We thank Dr Derek Smyth for the gift of the C fragment

of β -LPH used in these studies. Met-enkephalin and [D-Ala]²-Met-enkephalin were obtained from Dr Jaw-Ken Chang.

Note added in proof: Hahn *et al.*³¹ have found that N-allyl-Leu-enkephalin has antagonist properties in the guinea pig ileum.

CANDACE B. PERT
DONALD L. BOWIE
AGU PERT

Section on Biochemistry,
Adult Psychiatry Branch,
National Institute of Mental Health

JOHN L. MORELL
ERHARD GROSS

Section on Molecular Structure,
Reproduction Research Branch,
National Institute of Child Health and Human Development
Bethesda, Maryland 20014

Received 18 May; accepted 12 July 1977.

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Subcellular localisation of leucine-enkephalin-hydrolysing activity in rat brain

THE naturally-occurring pentapeptides leucine- and methionine-enkephalin which exhibit agonist activity at the opiate receptor¹ are subject to rapid deactivation by tissue homogenates^{2,3}. The primary mode of degradation has been demonstrated to be cleavage of the Tyr-Gly amide bond^{4,5}. It is not surprising, therefore, that the opiate actions of the exogenously administered peptides are transient⁶⁻⁹ and the biological half life of the peptides is very short. We have previously studied degradation of the enkephalins in relatively crude preparations and we present here a study of the subcellular localisation of enkephalin-hydrolysing activity (EHA) in rat brain. We found a heterogeneous distribution of EHA in rat brain subfractions and evidence for enzyme heterogeneity. Opiate receptors are apparently functionally unrelated to the site of EHA in synaptic membranes.

Rat brain subcellular fractions were obtained from whole rat brain, excluding medulla and cerebellum, by the method of Whittaker¹⁰. Blood was obtained by cardiac puncture and

Table 1 Enkephalin-hydrolysing activity in rat plasma, CSF and unlysed brain subfractions

Tissue (fraction†)	Enzyme specific activity*	Total protein (mg)
Plasma	2.41 ± 0.31 (6)	39.2 ± 7.45 ml ⁻¹
CSF	2.00 ± 0.85 (10)	0.54 ± 0.26 ml ⁻¹
Brain soluble (S ₃)	30.54 ± 5.57 (5)	36.05 ± 5.41
Nuclear (P ₁)	7.51 ± 1.22 (5)	11.82 ± 1.57
Microsomal (P ₃)	3.29 ± 1.11 (5)	24.38 ± 5.63
Myelin (A)	0.96 ± 0.91 (5)	10.63 ± 3.07
Synaptosomal (B)	7.75 ± 1.47 (5)	15.80 ± 2.40
Mitochondrial (C)	4.84 ± 1.76 (5)	21.19 ± 5.71

Values are mean ± s.d.; number of determinations in parentheses. The assay mixture, containing [3,5-³H]-Tyr-Leu³-enkephalin (1 μCi, final concentration 10⁻⁴ M), 0.5 M Tris-HCl buffer pH 7.4 (0.1 ml), and the preparation to be assayed (50-200 μg, protein), was diluted with distilled water to a final volume of 0.5 ml. The mixture was incubated at 37 °C for 15 min and an aliquot (0.2 ml) taken and added to ice cold methanol (0.1 ml). After separation of the denatured proteins by centrifugation, an aliquot (0.05 ml) of the supernatant was applied in a narrow band to the foot of a silica gel thin-layer plate (Kieselgel-GF₂₅₄, 5 × 20 cm, Merck) together with authentic samples of tyrosine and Leu³-enkephalin. After development using chloroform-methanol-acetic acid-water (45:30:6:9, v/v) as solvent, the markers were visualised with ninhydrin (1%, w/v in acetone). Zones corresponding to the substrate and product were scraped and the silica suspended in a mixture of water (2 ml) and PCS (5 ml, Amersham-Searle). Radioactivity was determined using a Packard 2450 scintillation counter. Protein concentrations were determined by the method of Lowry *et al.*¹¹.

*Enzyme specific activity expressed as nmol enkephalin hydrolysed per mg protein per min.

†Letters in parentheses refer to the classification of subcellular fractions as used by Whittaker¹⁰.

cerebrospinal fluid (CSF) by puncture of the cisternum magnum of rats. The EHA was assayed using [3,5-³H]-Tyr-Leu³-enkephalin (Radiochemical Centre, Amersham) as substrate, by either determining the rate of formation of [3,5-³H]-tyrosine or the rate of disappearance of the substrate after thin-layer chromatographic separation of the two tritiated species.

The pH optimum for EHA in plasma, synaptosomes and brain high speed supernatant was pH 7.2. Significant EHA was found in all subcellular fractions (Table 1). Plasma, in contrast to CSF, was found to be a potent source of EHA. This is consistent with the results of studies reporting detection of enkephalin-like activity in CSF (refs 12, 13). The soluble fraction (S₃) of rat brain was also a good source of EHA. Of the particulate fractions examined synaptosomal (B) and nuclear (P₁) fractions showed highest enzyme specific activity. The even distribution of EHA in a crude membrane fraction (P₂) from various rat brain regions shown by Meek *et al.*⁵ would be expected from the considerable EHA associated with the mitochondrial fraction as this activity would contribute to a high background level of EHA of other than synaptosomal origin.

In view of the putative role of the enkephalins as inhibitory neurotransmitters or neuromodulators, it was of particular interest to explore the localisation of the synaptosomal enzyme. The synaptosomal fraction (B) was lysed by suspension in water for 30 min at 0-1 °C. The lysate was then fractionated on a discontinuous sucrose gradient as described by Whittaker¹⁰. The activity of the subfractions obtained is shown in Table 2. Some soluble enzyme was released which possessed similar activity to that obtained in the soluble fraction from whole brain. The synaptosomal

Table 2 Enkephalin-hydrolysing activity in rat brain lysed synaptosomal subfractions

Fraction (fraction†)	Enzyme specific activity*	Total protein (mg)
Soluble (O)	35.26 ± 12.0 (4)	2.49 ± 1.33
Vesicular (D, E)	0.91 ± 1.00 (4)	1.30 ± 0.56
Synaptosomal membranes (F, G, H)	2.62 ± 0.96 (4)	7.40 ± 4.93
Intra-terminal mitochondria (I)	3.26 ± 1.19 (4)	2.20 ± 1.70

Values are mean ± s.d.; number of determinations in parentheses. Assay methods as for Table 1.

*Expressed as nmol enkephalin hydrolysed per mg protein per min.

†Letters in parentheses refer to classification of Whittaker¹⁰.

mitochondrial fraction also possessed activity similar to that found in the mitochondrial fraction from whole brain. The EHA associated with the vesicular fraction was low whereas significant EHA was associated with the synaptosomal membrane fractions (F, G, H) and this latter activity may have a significant role in terminating the physiological action of the enkephalins.

Simantov *et al.*¹⁴ have shown that synaptosomes contain the highest levels of enkephalin activity and this study shows that substantial EHA is present in intact synaptosomes, so it follows that enkephalins must be protected from hydrolysis in some way, possibly by sequestration within vesicles. This would be consistent with the evidence presented here and analogous to the subcellular storage and inactivation of acetylcholine¹⁰.

It was of interest to examine the sensitivity of EHA in brain subcellular fractions to various reagents in an attempt to cast some light on the uniformity of enzyme activity present. The results are presented in Table 3. Rat brain EHA was uniformly inhibited by leucyl-β-naphthyl-

Table 3 Effect of various reagents on rat brain and plasma enkephalin-hydrolysing activity

Tissue (fraction)	Leucyl- β -naphthylamide	EDTA*	<i>p</i> -Chloromercuribenzoate
Plasma	41	14	0
Brain soluble (S ₃)	79	47	90
Synaptosomal (B)	92	1	90
Mitochondria (C)	62	15	93
Soluble (O)	82	0	19
Synaptic vesicles (D, E)	100	100	42
Synaptosomal membranes (F, G, H)	71	0	47
Intra-terminal mitochondria (I)	78	16	92

Values are % inhibition. Final inhibitor concentration was 0.5 mM. Tissue classification as in Tables 1 and 2.

*Adjusted to pH 7.4 with NaOH.

amide (0.5 mM). The patterns of inhibition observed with EDTA and *p*-chloromercuribenzoate did not run in parallel suggesting considerable enzyme heterogeneity in the fractions studied.

In view of the structural similarities demonstrated^{15,16} between the enkephalins and standard opiates it seemed possible that the latter could be competitive inhibitors of EHA. Of a series of compounds tested (buprenorphine, etorphine, fentanyl, ketocyclazocine, methadone, morphine, nalorphine, naloxone, pentazocine and pethidine) representing a wide range of structures and activities, only etorphine, fentanyl and methadone showed any inhibitory properties (about 50% at 0.66 mM, the highest concentration tested). In the light of the high affinity that the majority of these compounds demonstrate for the opiate receptor, it can be concluded that the pharmacological receptors and the sites of enzyme activity in synaptic membranes are functionally unrelated.

In summary, a heterogeneous distribution of EHA has been observed in rat brain subfractions. The demonstration of activity in the synaptic membrane fraction and the low level of activity found in the synaptic vesicle fraction are observations not inconsistent with the putative role of the enkephalins as neurotransmitters or neuromodulators.

A. C. LANE

M. J. RANCE

D. S. WALTER

Reckitt and Colman Pharmaceutical Division,
Dansom Lane,
Hull, UK

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Unconventional serotonergic excitation in *Aplysia*

EXCITATORY potentials of long duration have been observed in vertebrate¹⁻³ and invertebrate^{4,5} neurones. In *Aplysia*, Gerschenfeld and Paupardin-Tritsch⁴ have found that 5-hydroxytryptamine (5-HT) can produce two kinds of long excitatory responses (A' and α) with different ionic mechanisms. The A' response was described as an increase in sodium conductance. The response became smaller as the cell was depolarised, and was extrapolated to a reversal potential of 0 mV. In contrast, the α response was attributed to a decrease in potassium conductance, since it had a reversal potential near -80 mV and became larger as the cell was depolarised. Krnjevic *et al.*³ and Weight and Votava¹ have hypothesised a similar mechanism for the long excitatory potentials which they observed in cat cortical neurones and frog sympathetic ganglia, respectively. Using the voltage clamp technique we observed a prolonged excitatory response to 5-HT, the characteristics of which are unlike either of those described previously. At hyperpolarised levels it resembles the A' response, becoming smaller with depolarisation, but at depolarised potentials the amplitude of the response dramatically increases.

Long excitatory responses with this characteristic could be recorded in cells of the left caudal quarter of the abdominal ganglion and in unidentified neurones of the buccal ganglia of *Aplysia californica*. The ganglion was pinned out and bathed in a solution of artificial seawater at pH 7.8. Cells were impaled with a glass capillary microelectrode filled with 1.5 M KCl. Membrane potential was clamped by the single microelectrode method of Wilson and Goldner⁶. An iontophoretic electrode containing a saturated solution of 5-HT was positioned extracellularly, and adequate bias current was applied to prevent leakage of the transmitter. To eject the 5-HT, standard pulses of positive current were passed through the electrode at a constant frequency of about once every 5 min. At this rate, desensitisation would not occur. It was ensured that the amplitude of the response was constant and that manipulation of membrane potential and the perfusion of seawater solutions would not alter the response. Since membrane potential was held constant by the voltage clamp, a depolarising response is expressed as inward current.

The membrane potential dependence of the long excitatory response is illustrated in Fig. 1a and b. As the cell is depolarised from -80 to -30 mV, the current response decreased in amplitude. Above -30 mV, the current response dramatically increased in size. In other cells this dramatic increase occurred at potentials as negative as -40 mV. Additionally, at depolarised levels, the current was prolonged and reached a maximum amplitude more slowly. The response seems to be comprised of two components: a linear component (1 in Fig. 1b) at hyperpolarised levels and a nonlinear component (2 in Fig. 1b) which begins to influence the response between -40 and -50 mV. This nonlinear component was observed in over 80% of the cells in which a prolonged excitatory serotonergic response was recorded (25 cells). In each case where component 2 was seen, it was as distinct and prominent as the sample shown in Fig. 1b.

Occasionally, the long response to 5-HT was accompanied by an excitatory response with a short time course (the A response of Gerschenfeld and Paupardin-Tritsch⁴). This fast response behaved in a totally conventional manner. As reported by Gerschenfeld and Paupardin-Tritsch⁴, the response became smaller with depolarisation and seemed to have a reversal potential near 0 mV. At potentials where the prolonged response was enlarged, the fast response in the same cell was extremely reduced in amplitude. This was

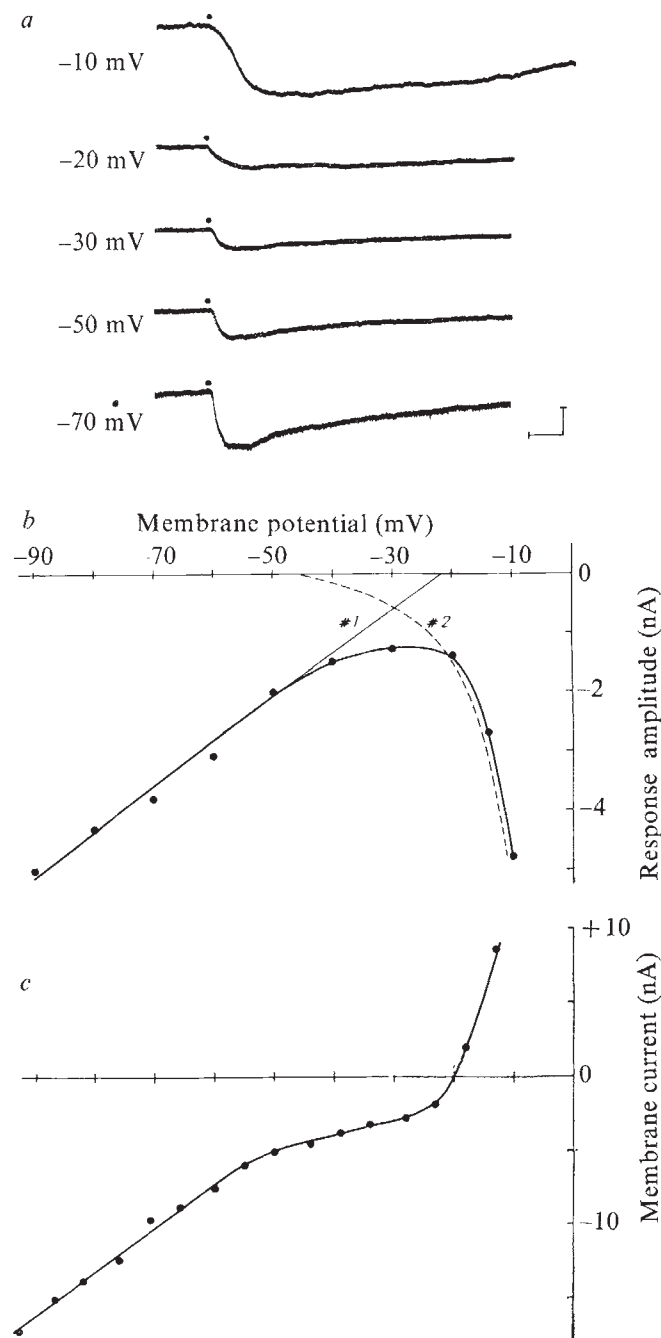


Fig. 1 *a*, Prolonged excitatory response to 5-HT. Cell membrane potential was voltage clamped and current was recorded. Ionophoretic pulse was applied at the dot. At -10 mV the response is prolonged and greatly enhanced. Calibration: 2 nA, 24 s. *b*, Plot of membrane potential against response amplitude for the same cell as in *a*. The response seems to be composed of two components: a linear component, labelled 1 and a non-linear component, labelled 2. *c*, Current-voltage relationship for the same cell as in *a* and *b*. Note the correspondence between the increase in the conductance of the cell and the enhanced response to 5-HT.

taken as evidence that the enhanced component 2 of the long response was not simply an artefact of the voltage-clamping system, for if it were, the short response would be equally enhanced.

A comparison of the current-voltage relationship of the cell (Fig. 1*c*) and the amplitude-potential relationship of the response to 5-HT (Fig. 1*b*) shows that the 5-HT response increased dramatically at the same potential where the membrane conductance increased. In the illustrated

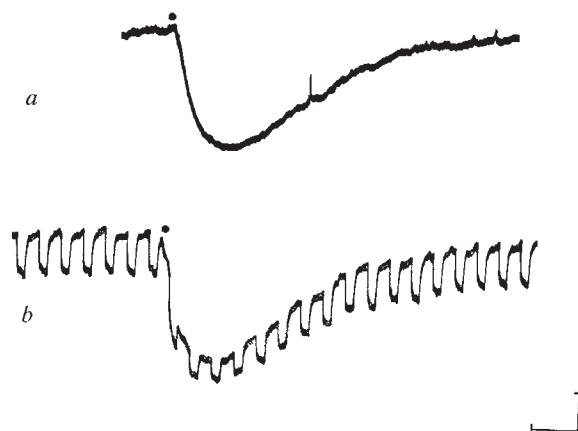
cell this point was approximately -20 mV (compare Fig. 1*b* and *c*). We considered the possibility that the anomalous response to 5-HT at depolarised levels (component 2) could be due to a reduction in the potentiated membrane conductance at these potentials. Figure 2 shows the results of conductance measurements before and during a response to 5-HT. While the potential of the cell was clamped to -12 mV, hyperpolarising command pulses were applied to the cell every 20 s. During the response to 5-HT the current change produced by these pulses was reduced, indicating an apparent conductance decrease. At membrane potentials more negative than -30 mV, this reduction in membrane conductance was not observed. In fact, there was either a small increase in conductance or, often, no measurable change at all in the membrane conductance of the cell during the response at -80 mV.

The apparent decrease in membrane conductance at potentials above -30 mV could be due to a decrease in potassium or chloride conductance. Preliminary ion substitution experiments show that this is not the case. In both high (30 mM) and low (1 mM) extracellular potassium, the response was unaffected. Decreasing extracellular chloride concentration to 77 mM was also without effect on the excitatory response to 5-HT. Figure 3*a* shows a typical ion substitution experiment. This particular experiment illustrates that elevation of extracellular potassium does not alter the response.

The apparent decrease in conductance could also be due to a voltage-sensitive increase in inward sodium current. In Fig. 1*b*, the line labelled 2 can represent such a current. As the cell is depolarised, the inward current is enhanced. Summing this component of the response with the current produced by a voltage-independent conductance change (1 in Fig. 1*b*) would result in the observed response. Since component 2 would be reduced during a hyperpolarising command, there would be an apparent conductance decrease. Such a voltage-sensitive sodium or calcium conductance has been shown to underline the regenerative slow wave potentials and negative resistance region of bursting cells⁷. This possibility is supported by ion substitution experiments. Replacement of extracellular sodium with sucrose consistently attenuated both components of the response, suggesting that the response is in some way dependent on sodium ions (Fig. 3*b*).

It is likely that the unconventional response shown here is actually the A' response of Gerschenfeld and Paupardin-Tritsch⁴, which was attributed to an increase in sodium conductance. Since their observations were made in an unclamped cell, rectification prohibited their recording at

Fig. 2 *a*, Long excitatory response to 5-HT, administered at the dot. Membrane potential was voltage clamped to -12 mV. *b*, Hyperpolarising voltage commands applied before and during an identical response to 5-HT. The current change produced by the voltage commands is reduced during the response. Calibration: 2 nA, 48 s.



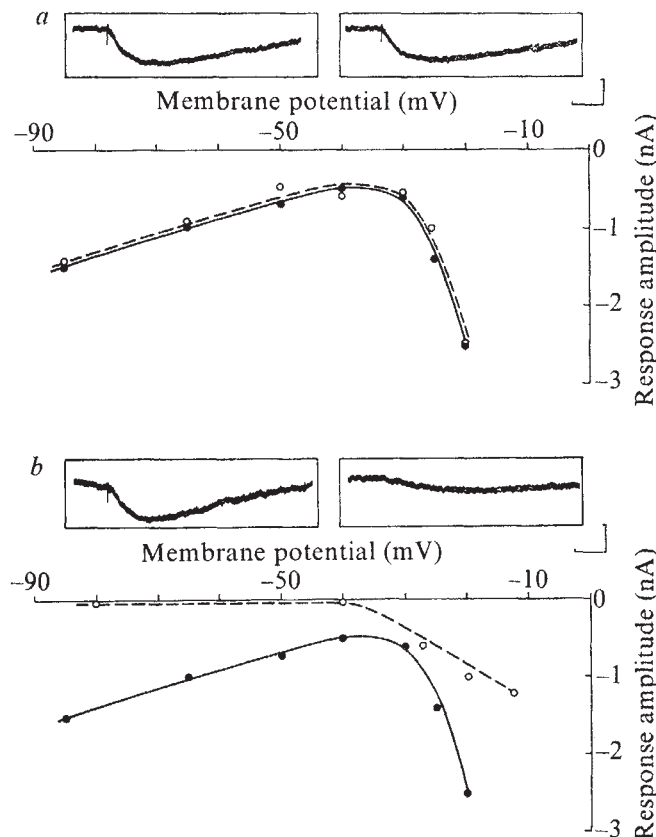


Fig. 3 Effect of ion substitutions on the long excitatory response to 5-HT. The responses shown in *a* and *b* were obtained from the same cell. *a*, Sample responses in normal (left) and 30 mM K^+ seawater (right) were recorded while the cell was voltage clamped to -20 mV. Calibration: 2 nA, 24 s. A plot of membrane potential against response amplitude illustrates that high potassium seawater ($\circ$) does not alter the responses obtained in normal seawater ($\bullet$). *b*, Sample responses at a membrane potential of -20 mV were greatly attenuated by Na^+ -free seawater, in which sucrose was added to maintain isotonicity. Calibration: 2 nA, 24 s. A plot of membrane potential against response amplitude illustrates that the Na^+ -free seawater ($\circ$) reduced the response at all membrane potentials. $\bullet$, Normal seawater.

very depolarised levels⁴. Fig. 1*b* shows that component 2 of the response would be missed unless potentials above -30 mV were sampled. Extrapolation of the curve consisting of only the points below -30 mV would indicate a reversal potential near -20 mV which is similar to that of conventional, sodium-dependent response^{4,8}. It is unlikely that the response we have observed is due to the stimulation of a combination of receptors which produce an increase in sodium conductance and a decrease in potassium conductance (A' and α , respectively). Our second component can first be seen to influence the current response near -40 mV and seems to have no effect on the response at more hyperpolarised potentials. Also the response is insensitive to changes in extracellular potassium concentration.

Our data show an unconventional long excitatory response to 5-HT. The response at hyperpolarised potentials behaves simply as an increase in sodium conductance, whereas at depolarised potentials the current is enhanced and accompanied by an apparent decrease in membrane conductance. This unusual component may be due to serotonergic activation of a voltage-sensitive sodium conductance, whereas conventional synaptic and iontophoretic responses result from voltage-independent conductance changes. The response we described represents a new type of neurotransmitter action: the induction of voltage-sensitive ionic channels.

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T. C. PELLMAR
W. A. WILSON

Department of Physiology and Pharmacology,
Duke University Medical Center,
and Epilepsy Research Laboratory,
Veterans Administration Hospital,
Durham, North Carolina 27705

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Light-induced fluctuations in membrane current of single toad rod outer segments

VERTEBRATE photoreceptors respond to illumination with a reduction in the steady current of Na ions which in darkness flows inwards across the outer segment membrane; this results in hyperpolarisation of the cells¹. The light responses of the receptors can be studied by measuring extracellular voltage gradients² or by intracellular recording³, but these methods can only provide information averaged over many photoreceptors owing respectively to high extracellular conductivity and to the presence of electrical coupling between cells^{3–6}. This averaging and the presence of dark voltage noise in photoreceptors^{6,7} have prevented observation of the electrical effects of individual photoisomerisations. To try to record these elementary events and to localise the source of the dark noise, we have developed a method for recording the membrane current of a single rod outer segment. The technique is based on that used by Neher and Sakmann⁸ on muscle fibres.

Small pieces of retina were isolated from dark-adapted toads, *Bufo marinus*, and maintained in previously oxygenated toad Ringer⁹. On the stage of an inverted microscope fitted with an infrared image converter, a single rod outer segment was drawn under visual control into a close-fitting suction electrode containing Ringer (Fig. 1). The resistance of the suction pipette was initially 1–2 M Ω and rose to 4–10 M Ω with an outer segment in place. A current sensor between the pipette and a reference electrode in the bathing solution recorded the bulk of the membrane current flowing across the region of outer segment within the pipette. Recording resolution was limited by thermal noise in the leakage resistance of the space between the electrode wall and the outer segment, and to measure transient currents of the order of picoamps the bandwidth was normally restricted to 5–10 Hz.

Flashes of light evoked net outward photocurrents which were graded with stimulus intensity. As in previous extracellular² and intracellular^{3–5,9,10} measurements, the response amplitude saturated with increasing intensity in a hyperbolic manner. The half-saturating photon density was about 1 photon μm^{-2} for 'red' rods stimulated with 500 nm light, comparable with results obtained with intracellular voltage recording⁴, and the maximum photocurrent obtainable from a cell corresponded closely to complete suppression of its dark current, with values for different cells ranging up to 23 pA. Although intracellular voltage responses to bright

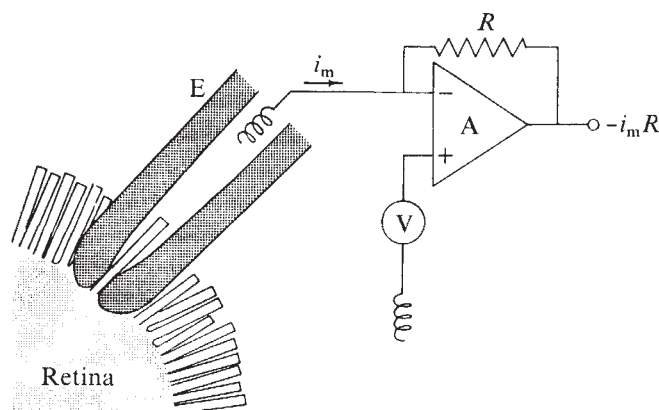


Fig. 1 Arrangement for recording membrane current of a rod outer segment. A suitably oriented outer segment was drawn by gentle suction into a close-fitting glass electrode, E, with fire-polished and vinyl-coated tip (Stoner-Mudge resin, Mobil). Membrane current, i_m , was recorded with amplifier A, a Function Modules model 380K, selected for low noise⁸. V, variable voltage used to compensate differences in electrode potentials and to measure electrode resistance. Amplifier output, $-i_m R$, was further amplified and recorded on a chart recorder and an FM tape recorder. Feedback resistance, R, 100 M Ω .

flashes display a prominent initial transient component^{4,9}, no such phase has been seen in the membrane current response from the outer segment. This may indicate that the voltage-sensitive channels generating the transient are not located in the outer segment. The dim flash responses were often considerably slower than the corresponding responses observed previously with internal electrodes⁴, with times to peak of 2–6 s. The reason for this is not clear, but it might result from our procedure of cutting the retina into small pieces.

Responses of a rod to a series of dim flashes of fixed intensity applied locally on its outer segment are shown in Fig. 2. There was considerable variation in response amplitude and four flashes seem to have failed to elicit a response. Measurement of the peak amplitude gave a mean of 2.0 pA and a variance of 2.2 pA², which, assuming a Poisson distribution, indicates an event size of 1.1 pA and

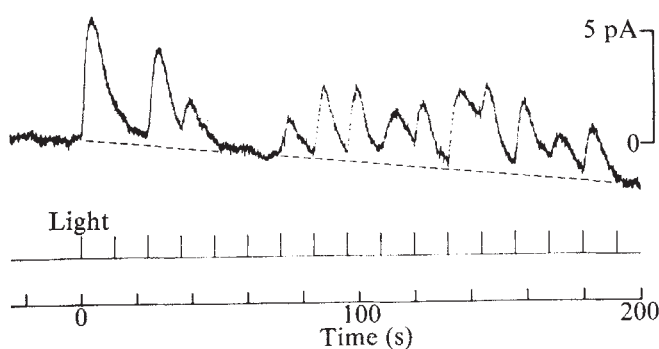


Fig. 2 Responses of a 'red' rod outer segment to brief flashes of blue-green light delivered at intervals of 12 s. Membrane current above, outward current upward, bandwidth 5 Hz; stimulus monitor below. Stimuli were local slits of light slightly larger than the 40 μm length of outer segment in the electrode. Intensity of the 500 nm stimulus at the preparation was estimated from readings of the unattenuated light made with a Tektronix digital irradiance meter and from *in situ* calibrations of the neutral density filters made with a photon counter (Princeton Applied Research, Quantum photometer). The flash width at half amplitude was 18 ms, and the estimated mean photon density was 7.9×10^{-2} photons per μm^2 per flash. The effective collecting area of the portion of the outer segment in the electrode was estimated as 12.5 μm^2 from measurements of cell dimensions, assuming an axial pigment density of 0.016 μm^{-1} and a quantum efficiency of isomerisation of 0.65.

a mean of 1.8 events per response. If a correction were made for nonlinear summation, the estimated event size would increase somewhat, and the mean number of events per flash would correspondingly decrease. The intensity of the light applied to the preparation was determined from photometer and photomultiplier readings as 0.079 photons μm^{-2} which was estimated to cause a mean of 0.99 photoisomerisations in the portion of outer segment within the suction pipette. This number of events is in reasonable agreement with the value of 1.8 estimated from the current fluctuations.

Figure 3 illustrates responses of the same rod to steady lights of increasing intensity. The lowest intensity (a) gave a response which rose with a considerable delay after the shutter opened and which fluctuated and then disappeared

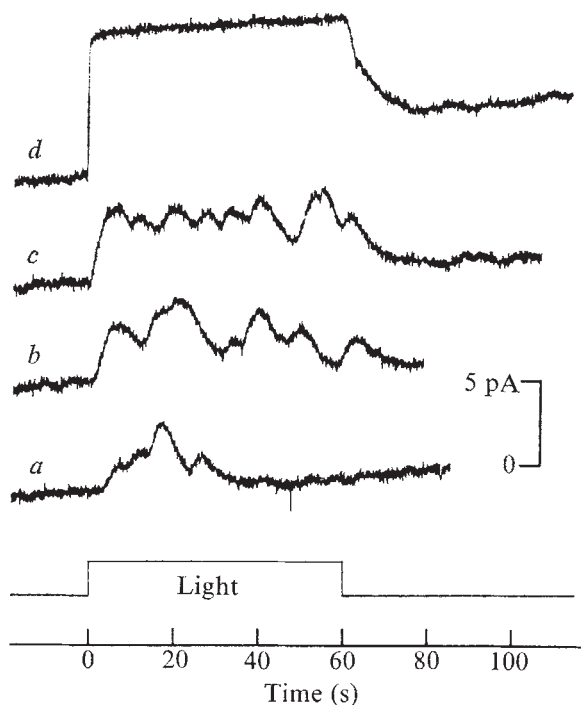


Fig. 3 Responses of the outer segment of Fig. 2 to steady lights of increasing intensity. Upper traces (a–d): membrane current, bandwidth 10 Hz; lower trace: stimulus monitor. For each response the intensity of 500 nm light was (photons $\mu\text{m}^{-2} \text{s}^{-1}$): a, 6.1×10^{-3} ; b, 2.0×10^{-2} ; c, 3.6×10^{-2} ; d, 30.

before the shutter closed. Two higher intensities (b, c) gave larger responses still marked by fluctuations, but a saturating light (d) gave a smooth response. The light for trace (a) had an intensity of 0.0061 photons $\mu\text{m}^{-2} \text{s}^{-1}$, which should on average have caused about 0.076 photoisomerisations per second in the recorded region of outer segment. On this basis the number of isomerisations expected in a 60-s stimulus would have been 4.6 ± 2.1 . Inspection of trace (a) shows four discrete deflections although it is possible that more events may have occurred. The absence of a response in the later part of the trace presumably resulted from the absence of any isomerisations during that period. In trace (b) the mean response during illumination was 2.5 pA and the variance was 0.7 pA². Ignoring nonlinear summation of elementary events and taking a shape factor¹¹ of 0.68 gives the amplitude of the elementary event as 0.42 pA, in rough agreement with that obtained from the flash run. The mean number of simultaneous events in trace (b) would then have been 6.0, which, with an integration time⁹ of 8 s (from the flash responses of Fig. 2), corresponds to 0.75 events per second, as compared with a rate of 0.25 photoisomerisations per second estimated from the light intensity and cellular dimensions.

Assuming that the driving potential on the light-

sensitive conductance is 40 mV (refs 1, 3, 10), the observed current event of about 1 pA would correspond to a photon-induced conductance decrease of 25 pS. This is comparable to the conductance of an acetylcholine channel^{11,12}, but several times larger than that of a sodium channel in nerve^{13,14}. An elementary current event of 1 pA together with the measured flash sensitivity of 700 μ V per photoisomerisation with diffuse light⁴ indicates that the resistance of an individual rod would be about 700 M Ω in the absence of coupling to other cells.

A noticeable feature of the presumed responses to single photons was their rounded shape. In contrast, the opening and closing of single channels in other preparations leads to rectangular-shaped changes in conductance^{8,15}. This may imply that a large number of channels are affected by each photoisomerisation or that a single channel fluctuates so rapidly between open and closed states that only the average behaviour is observed.

Responses to bright lights, such as that in Fig. 3d, occasionally showed suppression of the dark noise, similar to the effect seen with intracellular recordings^{6,7}. In one cell the variance measured in the band 0.2 to 5 Hz was 0.050 pA² in darkness and 0.017 pA² during a light which gave a saturating response of 13 pA. The difference power spectrum was broadly consistent with a Lorentzian of half power frequency 1.5 Hz. It is not yet clear whether such dark current noise arises from conductance fluctuations in the outer segment or from fluctuations in intracellular voltage arising from a noise source elsewhere in the cell.

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Note added in proof: It has come to our attention that a similar method of recording from rod outer segments has been reported by Jagger¹⁶.

K.-W. YAU
T. D. LAMB
D. A. BAYLOR

Department of Neurobiology,
Stanford University School of Medicine,
Stanford, California 94305

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Transport mechanism operating between blood supply and osteocytes in long bones

WE show here that cyclic loading, as naturally occurring in long bones, produces a flow of liquids through canaliculi. The magnitude of such a stress-induced flow is so great that it significantly increases the efficiency of the transport mechanism operating between blood supply and osteocytes.

The present state of knowledge, according to the curricula in biology and medicine, describes the mechanism of supply of nutrients to Haversian bone cells (osteocytes) and disposal of the waste products from the cells by the process of diffusion^{1,2}. Diffusion is a very powerful mechanism in other areas of the body where, first, there is a short distance between the vessel and the

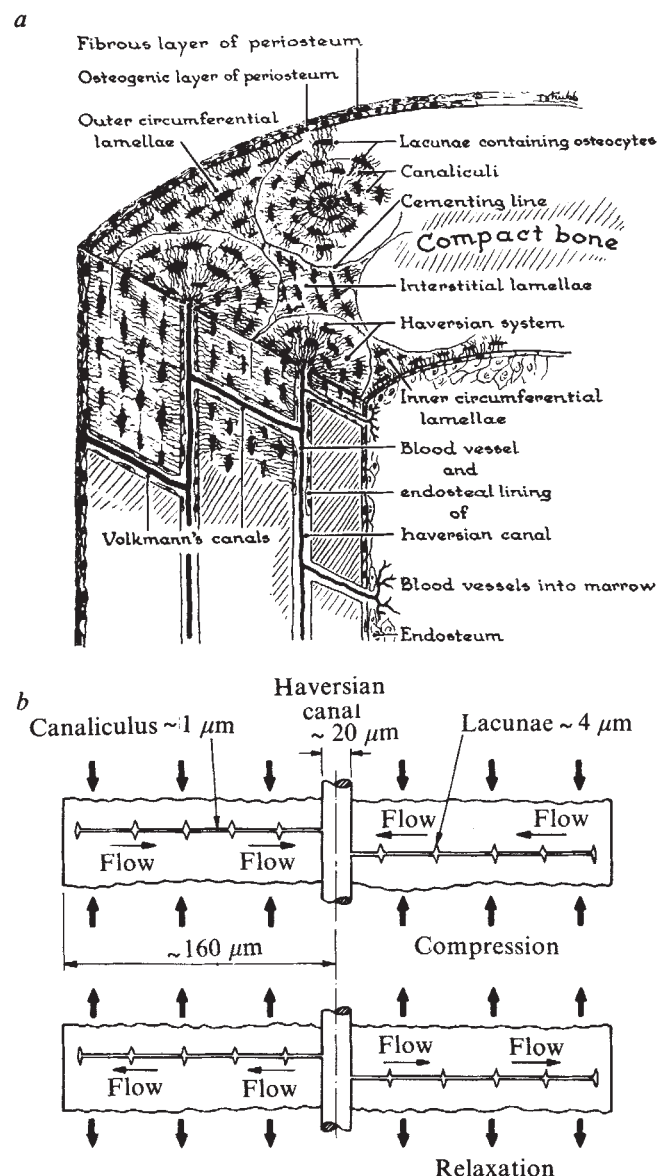
cell and, second, the cells are surrounded by fluid. The osteocytes of Haversian bone (Fig. 1a), however, are almost completely entombed in a calcified matrix except for the long and narrow canaliculi through which the nutrients and waste products must pass. Thus, diffusion is probably quite weak and it must be aided by another mechanism.

Assuming that bone is compressible and is loaded in compression, the volume of the canaliculus would decrease and thus pump the liquids out into the central vessel. After unloading, fresh nutrients will be drawn in from the Haversian canal into canaliculi (Fig. 1b). There is indeed much supporting evidence that cyclic loading of bone affects its mass; it grows thick and healthy. Immobilisation, bed rest or weightlessness, results in atrophy and in general loss of bone mass.

The proposed mechanism is examined here in greater detail. The lamellar structure of the Haversian system has been previously described and earlier studies established only that lamellae differ in the orientation of collagen fibres and perhaps in their mineral content. More observations were made recently by Vincent³, Ascenzi *et al.*⁴ and Boyde⁵. The pertinent information of these investigators may be summarised as follows.

Concentric cylinders of lamellae may differ from each other by their size, orientation of collagen fibres and ratio of mineral to

Fig. 1 a, Schematic representation of the microstructure of cortical bone¹. b, Simple model of stress-induced flow in the lacunar-canalicular system.



organic phases. Ascenzi *et al.*⁴ distinguished some of the lamellae as true lamellae, 5–7 μm wide, and some as interlamellar cementing zones, 1.5–2 μm wide. The latter displayed random orientation of collagen compared with the regular fibrous structures of lamellae.

Scanning electron micrograph of a fractured bovine bone treated with ethylene diamine, to remove the organic phase also revealed lamellar arrangements of phases in an osteon (Fig. 2). Similar observations were made on sectional specimens of bone treated with 95% hydrazine. It may reasonably be assumed that the empty spaces between the solid cylinders had a higher content of the organic phase, and any mineral phase present existed there in the classical form of discontinued crystallites which presumably dropped out on removal of the organic phase. Closer examination of the remaining mineral rings suggests a continuous polycrystalline structure (Fig. 3).

For the study of flow within the lacunar–canalicular network, organic material with suspended mineral was assumed to behave as a liquid, that is, capable of transmitting hydrostatic pressure. Thus, the Haversian system may be visualised as multiple concentric porous mineral cylinders separated by regions of liquid (Fig. 4). Such a structural arrangement can be easily analysed by a mathematical and physical model. It was also assumed that the pressure in the central cylinder would remain constant (equal to capillary blood pressure) and independent of flow through the canaliculi. The general concept and solution technique for axially loaded liquid-filled single and multiple concentric cylinders has been previously described in great detail (refs 6, 7).

After axial compressive loading of the model (Fig. 4), a hydrostatic pressure gradient is produced between the liquid regions. As flow occurs through the cylinders (canaliculi modelled as circular tubes), liquid on the inner side of the peak pressure moves radially inwards as in the simple model (Fig. 1*b*). Liquid on the outer side of the peak pressure, however, initially travels outwards and then, after $t = 0.0013$ s, inwards. Thus, in one-half of the loading cycle, the middle and outer areas of the model experience a two-way flow, whereas the inner areas only a one-way flow. This same pattern, opposite in direction, occurs after release of the load.

In both the simple and concentric cylinder models, it has been shown that cyclic stressing produces cyclic flows within the lacunar–canalicular network of the Haversian system. What remains to be shown is the order of magnitude of difference between the effect of the stress-induced flow and diffusion in transporting nutrients and waste products.

A set of calculations was performed for each liquid region to compare a common variable, the local displacement of a constant concentration, between a diffusion model and the stress-induced flow model for the period of 0.5 s, which approximates to one-half of a walking cycle, that is, a period of a normal loading of a long bone. The local displacement for the stress-induced flow model was calculated by assuming that the total volume change of that

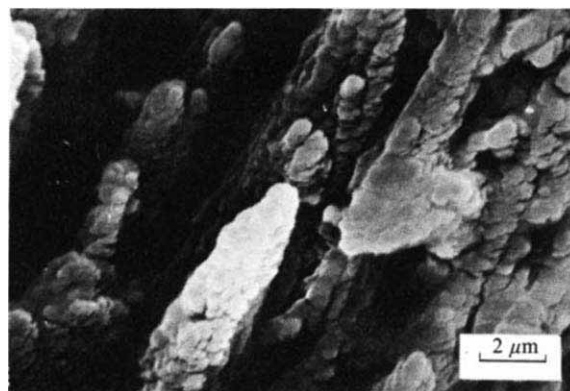


Fig. 3 Portion of a lamella of the osteon in Fig. 2 illustrating polycrystalline arrangement of the mineral phase.

region and its outer regions flowed through the canaliculi of that region.

For the diffusion model, a linear concentration gradient of the diffusing matter varying from 100% in the central liquid region to 10% in the outermost liquid region was assumed. One-dimensional diffusion in a semi-infinite medium was considered, that is

$$C = C_0 [1 - \text{erf}(x/2(DT)^{1/2})]$$

The estimate of the diffusion coefficient, $D = 10^8 \text{ cm}^2 \text{ s}^{-1}$, was based on the results of the *in vitro* bone diffusion experiments of Amprino⁸ and Lang *et al.*⁹. The local displacement for the diffusion model was the distance that the assumed local concentration moved in 0.5 s. The ratio of the local displacement of the stress model to that of the diffusion model as a function of radius is given in Table 1.

Table 1 Comparison of local displacement values for stress-induced flow and diffusion models

Liquid region	Radius (μm)	Ratio: induced flow/diffusion.
2	16	3,700
3	23	1,140
4	30	490
5	37	230
6	44	110
7	51	46
8	58	12

Although no direct calculation can be made relating fluid movement and diffusion in the stress induced flow model, it is felt that diffusion would be aided significantly by increased mixing caused by the very large relative motion of liquids through both the rough-walled canaliculi and the lacunae.

In addition to this very basic comparison, an examination of the pattern of the flow in Fig. 4 indicates that the reversed directions of flow in canaliculi during compression and relaxation cycle would produce additional mixing of liquids, thus aiding further the mechanism of diffusion. It is also reasonable to assume considerable mixing to occur in each lacunae until diffused product would reach most outwardly located osteocytes.

The mechanism of the radial motion of molecules through the canaliculi and lacunae is completely unknown. The osmotic behaviour of cell membranes and processes surely plays an important part. An approximation of the canaliculus to a round tube is also inaccurate. An electron microscope examination of the wall of the canaliculus reveals uneven and porous surfaces¹⁰. Thus, there may be many other factors affecting diffusion processes between the blood vessels and bone cells. This investigation demonstrates the existence of one very powerful aiding mechanism

Fig. 2 Fractured surface of a bovine osteon deproteinised with ethylene diamine.



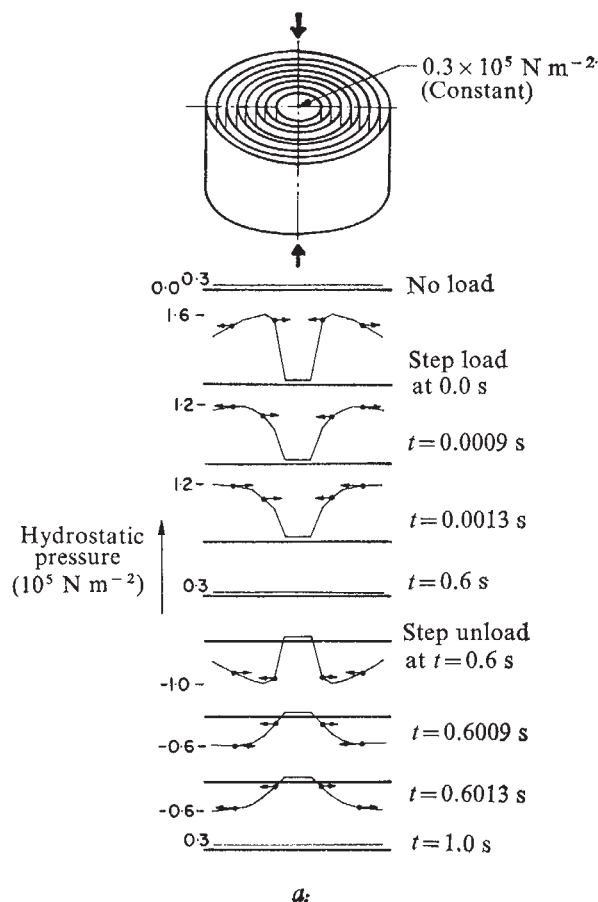


Fig. 4 Stress-induced flow in the lacunar-canalicular system modelled as a number of concentric cylinders separated by regions of liquid (detailed calculations in ref. 7).

which should not be neglected when considering transport of molecules through the canaliculi.

K. PIEKARSKI

University of Waterloo,
Department of Mechanical Engineering,
Waterloo, Ontario, Canada N2L 3G1

M. MUNRO

University of Cambridge,
Engineering Department,
Trumpington Road,
Cambridge, UK

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Unstable protein mediated ultraviolet light resistance in *Anacystis nidulans*

CYANOBACTERIA are believed to have been precursors to eukaryotes during Precambrian evolution¹. It is also suggested that the oxygen evolved as a result of photosynthetic activity in these organisms, several of which are obligate photoautotrophs, was the major contributor towards the transition from anaerobic to aerobic atmosphere^{1,2}. It is, therefore, reasonable to expect cyanobacteria to have evolved in an environment with a relatively high flux of

solar ultraviolet light in the absence of ozone shield. This, presumably, could be possible with the evolution of efficient protective mechanisms or repair systems effective against damage by ultraviolet light. It is therefore not surprising that these organisms possess an extremely efficient photo-reactivating repair system³. The presence of dark-repair in *Anacystis nidulans* has also been suggested by the isolation of mutants sensitive to ultraviolet light⁴. We present here physiological evidence for a dark-repair (or protective) system in this organism. A protein, unstable at least in the light, seems to be responsible for the resistance against lethal damage by ultraviolet light. This is observable in conditions of reduced photoreactivation achieved by a 24 h post-irradiation dark incubation, referred to here as 'dark-survival' following Asato, who showed that well within this period photoreactivability of damage by ultraviolet light is almost completely lost⁴.

Strain BD-1 *A. nidulans* was cultured and plated in synthetic medium, C-Mn, as described by Van Baalen⁵. The cells were grown in light (200 foot-candle (fc)) from tungsten lamps, at 35 °C in a shaker. Exponentially grown cells were used for all the experiments. The plates were incubated in the presence of 200 ± 10 fc of light from tungsten lamps and were maintained at 36 ± 1 °C in an incubator. After 5-6 d of growth the colony counts were taken.

Incubation of cells with chloramphenicol before exposure to ultraviolet light sharply reduced their dark-survival (Fig. 1). Almost the maximum reduction in the dark-survival at

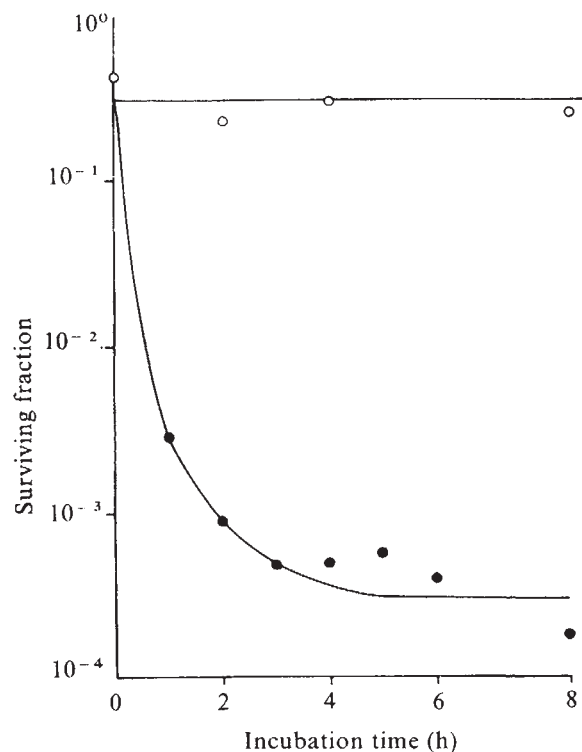


Fig. 1 Kinetics of survival by *A. nidulans* on exposure to ultraviolet light during incubation in light in the presence and absence of chloramphenicol. Exponential phase cultures of strain *A. nidulans* BD-1 at a concentration of 10^7 - 10^8 cells per ml without further washing were divided into two fractions. In one fraction chloramphenicol was added to a final concentration of $5 \mu\text{g ml}^{-1}$ (●). The other fraction contained no chloramphenicol (○). Both the fractions were incubated at 36 ± 0.5 °C in a water bath shaker. Light from tungsten lamps was incident at an intensity of 200 fc. At indicated time after the addition of chloramphenicol samples were diluted in the growth medium at 35 °C and spread on synthetic agar plates prewarmed to the same temperature. Plates with cells were exposed to 165 J m^{-2} of ultraviolet light (254 nm) from Philips TUV15W germicidal lamp at an intensity of $11 \text{ J m}^{-2} \text{ s}^{-1}$. The control and ultraviolet light exposed cells were kept in the dark for 24 h at 35 °C. After the dark-incubation all the plates were transferred to light (200 ± 10 fc) at 35 ± 1 °C. After incubation in light for 5-6 d the colony counts were taken.

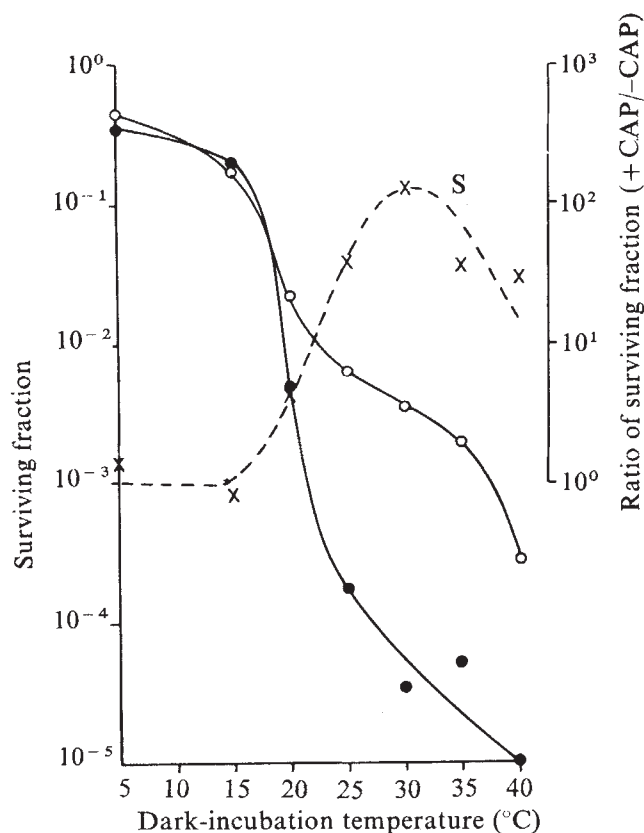


Fig. 2 Survival of *A. nidulans* on exposure to ultraviolet light when preincubated in visible light in the presence and absence of chloramphenicol (CAP), as function of post-irradiation dark-incubation temperature. Exponentially growing cells without further washing were incubated in light (200 fc) without (○) and with $5 \mu\text{g ml}^{-1}$ of chloramphenicol (●) for 3 h at $36 \pm 0.5^\circ\text{C}$ in water bath shaker. Control and treated cells were washed and resuspended in fresh growth medium at 35°C and 10 ml suspensions of each were immediately exposed to 330 J m^{-2} ultraviolet light. 1-ml aliquots of control and irradiated cells were transferred to thermally equilibrated tubes at indicated temperatures in the dark. After 24 h of dark-incubation at the respective temperatures the cells were diluted, plated and grown for colony counts as described in the legend to Fig. 1. The ratio of surviving fraction of untreated to chloramphenicol treated cells at the indicated temperatures is also shown (x).

35°C was achieved in about 3 h of chloramphenicol treatment. The extent of the dark-survival also depends on the post-irradiation temperature (Fig. 2). This dark-survival was significantly lower in the cells pretreated with chloramphenicol in the temperature range $20\text{--}40^\circ\text{C}$. The ratio of the surviving fractions of the untreated to the chloramphenicol treated cells was a function of the temperature of the dark-incubation, with a maximum at about 30°C . These results strongly suggest that some protein(s) normally present in the cell before the ultraviolet light exposure is involved in the dark-survival. The cells preincubated with chloramphenicol for 3 h or more are deficient in this protein suggesting its unstable nature. The higher survival at lower temperatures in Fig. 2 may be due to the arrest of (enzymatic) reactions leading to the loss of photoreversibility of the lesions which on subsequent incubation for growth in light may be removed by photoreactivation. When the chloramphenicol treated cells were exposed to photoreactivating light after ultraviolet light irradiation, their survival was comparable to untreated cells showing that the above protein(s) is not the photoreactivating enzyme. Incubation in the dark before ultraviolet light exposure is known to increase the dark-survival of this organism⁶. Chloramphenicol treated cells also showed a similar resistance when incubated in dark before ultraviolet light exposure. This unstable protein, therefore, may not have

a significant role in the development of the resistance of cells to ultraviolet light on preincubation in the dark.

The repair protein(s) described here is unstable in as much as the associated repair capacity is considerably reduced by the preirradiation treatment with chloramphenicol. In contrast, the same treatment does not measurably affect the photoreactivating capacity. A previous attempt to detect the presence of an unstable repair protein in yeast was unsuccessful⁷; in yeast pretreatment with nuclear specific protein synthesis inhibitor did not affect the liquid holding recovery⁷. In *Escherichia coli* an inducible repair protein synthesised after exposure of the cells to ultraviolet light has been detected⁸⁻¹⁰.

It is not clear whether this unstable protein has a role in unirradiated cells of *A. nidulans*. This organism may possess a constitutive or a visible light inducible repair pathway involving a protein which is independent of light for its action. Such a system could have a very important role in ensuring survival in an environment where photoreactivating system could not meet the challenge by a relatively high intensity of ultraviolet light. Regulation of repair processes by visible light has been suggested for eukaryotes^{11,12}. No specific mechanism is known.

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S. K. BHATTACHARJEE

Biology and Agriculture Division,
Bhabha Atomic Research Centre,
Bombay 400 085, India

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Detection of casein messenger RNA in hormone-dependent mammary cancer by molecular hybridisation

THE growth and development of the normal mammary gland are regulated by the complex interaction of both peptide and steroid hormones¹. Whereas some breast cancers retain the hormone-dependent characteristics of normal mammary tissue^{2,3}, other tumours lose this responsiveness and grow autonomously. Following the pioneering work of Huggins⁴ on the hormonal dependence of both experimental and human breast cancer, investigators seeking a prognostic test have studied the relationship of specific oestrogen receptors to mammary cancer^{5,6} to the response of the tumour to endocrine ablation^{7,8}, and to the levels of other steroid⁹ and peptide hormone receptors^{10,11}. A superior marker for hormone responsiveness would, however, be a measurable product of hormone action rather than the initial binding interaction. In the mammary gland, casein synthesis has been used as a specific biochemical marker of differentiated function and hormone responsiveness. In the studies reported here, a specific complementary DNA copy (cDNA) of rat casein mRNA has been utilised to study the effects of hormones on the expression of differentiated function in chemical carcinogen-induced mammary adenocarcinomas. It is shown that casein mRNA as detected by cDNA may be a useful indicator of hormone dependence in experimental breast cancer. The cDNA probe was synthesised using as a template a 15S casein mRNA fraction

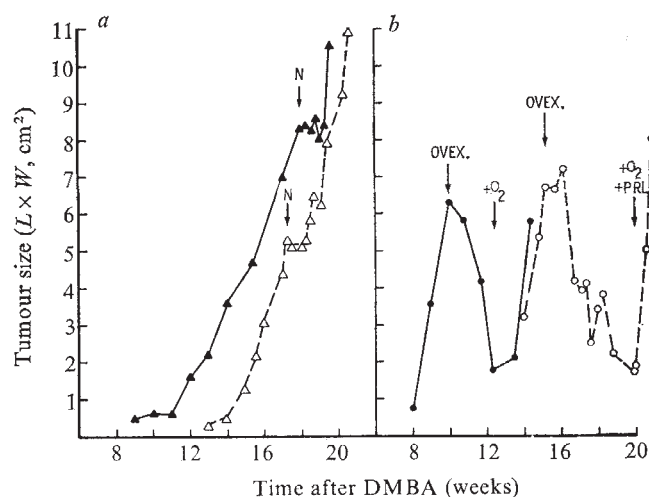


Fig. 1 Effect of hormonal treatment on the growth of DMBA-induced rat mammary carcinomas. Mammary adenocarcinomas were induced in 50-d-old female Sprague-Dawley rats by a single intubation of 20 mg DMBA. Tumours usually appeared 8–14 weeks after the administration of the carcinogen. *a*, Hormone dependence was assessed without ovariectomy by the single injection of an anti-oestrogen, nafoxidine 200 μ g s.c., given at the time indicated by the arrow. *b*, Hormone dependence of tumours was also determined by measuring the response to ovariectomy and the administration of hormones, either oestradiol benzoate (10 μ g every 3 d s.c. in sesame oil, ●), or oestradiol benzoate and prolactin (ovine, 35 IU mg⁻¹, (NIH-P-S12) 1 mg daily i.p., ○). Arrows indicate the time of ovariectomy (OVEX) followed by hormone administration.

purified 180-fold from a lactating RNA extract by chromatography on dT-cellulose and several precise sizing techniques^{12,13}. The template mRNA fraction represented greater than 90% casein mRNA, as estimated by specific immunoprecipitation of the total protein synthesised in the wheat germ assay, and by a careful analysis of its kinetics of hybridisation with the cDNA probe¹³. The specificity of the complementary DNA copy of the 15S casein mRNA has been demonstrated previously¹³. This cDNA probe

selectively hybridised to RNA from lactating tissue but not to rat liver poly(A)-containing RNA and the resulting hybrid displayed a high T_m characteristic of a well base-paired duplex. In addition, the rate of hybridisation of the casein-specific cDNA to various RNA preparations was directly related to the casein mRNA activity of these same preparations determined by a cell-free translation assay.

In these studies, 7,12-dimethylbenz(a)anthracene (DMBA)-induced rat mammary carcinomas were utilised as a model for hormone-dependent breast cancer^{3,4}. At least 80% of these tumours have been reported to respond to endocrine ablation and the levels of both oestradiol and prolactin receptors have been characterised previously¹¹. In addition, a limited number of autonomous mammary tumours induced in BALB/c mice have been screened for the presence of casein mRNA sequences. The amount of casein mRNA was quantified by analysing the rate of hybridisation of the complementary DNA with total RNA extracts of lactating and tumour tissue.

The characteristic growth response of the DMBA-induced rat mammary tumours to either ovariectomy and hormone administration or anti-oestrogen treatment are shown in Fig. 1. Ovariectomy resulted in a marked reduction in tumour size (Fig. 1*b*). Following either oestradiol administration or a combination of oestradiol and prolactin treatment, rapid growth was re-initiated. A single injection of nafoxidine, an oestrogen antagonist, also prevented the growth of hormone-dependent tumours for approximately 1 week without ovariectomy. (Fig. 1*a*). Following the return to the previous growth rate in the presence of the endogenous hormonal milieu, tumours were then removed and frozen before RNA extraction.

The hybridisation of the casein-specific cDNA to purified casein mRNA, RNA isolated from lactating tissue, and RNA isolated from some of the various hormone-dependent tumours is illustrated in Fig. 2. The 15S casein mRNA displayed a rate of hybridisation consistent with its high purity, and hybridised approximately 166 times faster than a total RNA preparation from 8-d lactating tissue. Both of these reactions occurred with the expected pseudo-first-order kinetics and proceeded to greater than 90% completion. Both the rates and extents of hybridisation of the

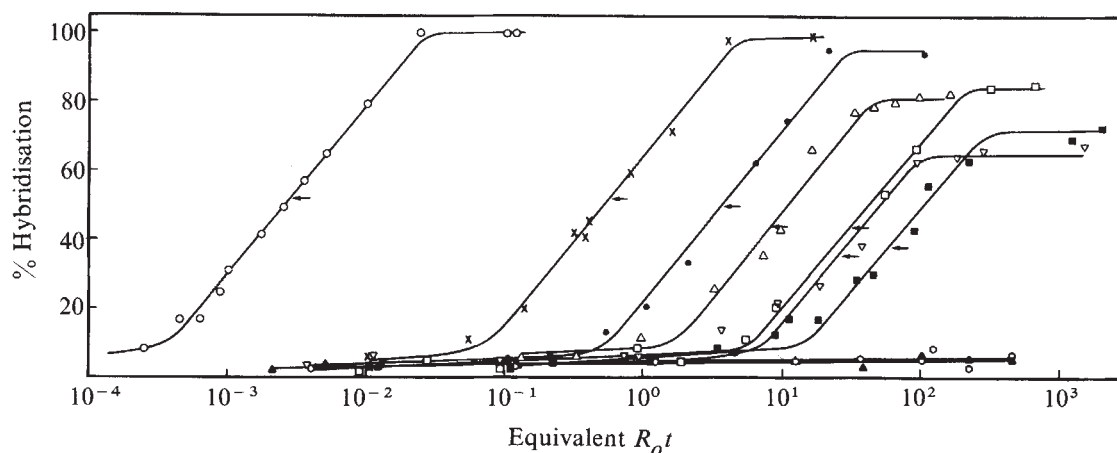


Fig. 2 Detection of casein mRNA sequences in hormone-dependent rat mammary carcinomas by molecular hybridisation. Tumours were excised, necrotic tissue removed and the remaining tissue rapidly frozen in liquid N_2 . The isolation of total cellular RNA by the direct homogenisation of pulverised, frozen tumour tissue in a phenol-SDS solution at pH 8.0 has been described previously¹². Before hybridisation all RNA extracts were treated with Proteinase K (25 μ g ml⁻¹ at 37 °C for 10 min) followed by a final phenol-CHCl₃ extraction at pH 8.0. RNA excess hybridisations were carried out in 0.6 M NaCl, 0.01 M HEPES, pH 7.0, 0.002 M Na₂ EDTA at 68 °C, and the extent of hybridisation was determined by S_1 nuclease as described previously¹³. The data are expressed as the percentage hybridisation compared with the log equivalent R_0t , the concentration of RNA (mol l⁻¹) times the time (s). The rates of hybridisation have been adjusted to the standard conditions of 0.18 M Na⁺ at 62 °C. The amount of DNA in the total nucleic acid extracts was determined by a standard di-phenylamine assay and the rate of hybridisation adjusted accordingly. DNA did not contribute to the extent of hybridisation observed in any of the tumours at the R_0t values assayed¹³. The following typical hybridisation curves are presented in this figure. Hybridisation of ³²P-cDNA with purified 15S rat casein mRNA (○), RNA isolated from 8-d lactating mammary tissue (×), RNA isolated from DMBA-induced tumours treated with oestradiol + prolactin following ovariectomy (●, △, ▴); treated with oestradiol alone following ovariectomy (○, ▽); and from tumours growing in a rat previously treated with nafoxidine (○, ▽).

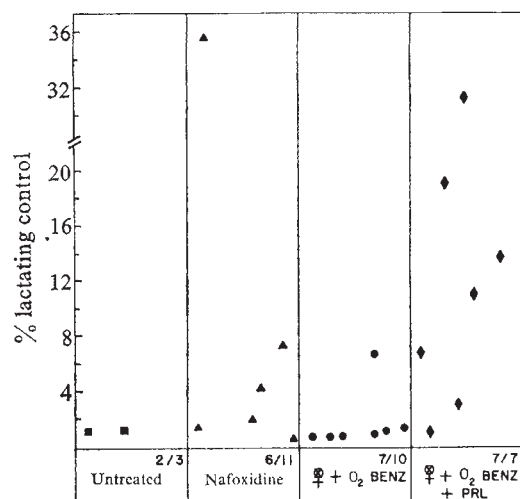


Fig. 3 Quantification of casein mRNA levels in hormone-dependent DMBA-induced rat mammary carcinomas. A total of 31 DMBA-induced tumours were assayed for the presence of casein mRNA by molecular hybridisation as described in Fig. 2. The tumours were divided into four groups based on the treatment received (see Fig. 1). The data are expressed as a percentage of an 8-d lactating RNA preparation, which was run as a control during each group of hybridisations. The lactating RNA contained approximately 0.52% of the total cellular RNA as casein mRNA, equivalent to approximately 80,000 molecules of casein mRNA per alveolar cell¹³. The number of positive tumours containing casein mRNA divided by the total number of tumours assayed in each treatment group is given as a fraction beneath each group.

tumour RNA preparations were less than those observed for the RNA preparation from lactating tissue. The maximum hybridisation observed at saturation or achieved by a R_{0t} (the concentration of RNA, mol l^{-1} , times the time, s) value of 1,000 for the casein mRNA containing tumour RNA extracts ranged from 25–100%. Any RNA extracts which displayed less than 20% hybridisation by a R_{0t} value of 1,000 were considered not to contain significant amounts of casein mRNA.

The data obtained from these hybridisation experiments are summarised in Fig. 3. Significant hybridisation was observed for 22 out of 31 (approximately 70%) of the RNA preparations isolated from the hormone-dependent tumours. The levels of casein mRNA found in the hormone-dependent tumour RNA preparations ranged from 0–36%

of those found in the 8-d lactating RNA preparation, which had been estimated previously to contain as many as 80,000 molecules of casein mRNA per alveolar cell¹³. The highest levels of casein mRNA were observed in tumours of ovariectomised rats treated with both oestradiol and prolactin and the lowest in tumours growing under endogenous hormonal stimulation. The amount of casein mRNA present in tumours of animals treated with both oestradiol and prolactin was also greater than the level found in animals treated with oestradiol alone. This result is consistent with the role of prolactin in the induction of casein synthesis and casein mRNA in normal mammary tissue¹⁴. A wide range was observed, however, in casein mRNA sequence levels in the different primary DMBA-induced tumours even within a given treatment group. A similar variability in the levels of prolactin and oestradiol receptors has also been reported in these DMBA-induced tumours¹¹. This variability is most likely explained by the heterogeneity of cell types that exists within these tumours. Some cells may contain prolactin receptors and display the normal hormonal response, whereas others may either lack prolactin-binding sites or display an altered response. Two such distinct populations of cells have actually been identified in DMBA-induced mammary tumours by autoradiographic localisation of prolactin receptors using ¹²⁵I-prolactin¹⁵.

Whereas the majority of the DMBA-induced mammary adenocarcinomas in Sprague-Dawley rats are dependent on hormones for growth, those which appear in BALB/c mice are characteristically hormone-independent. The molecular basis for this autonomy and the difference in response of related species to the same chemical carcinogen are not well understood. Several transplantable mouse mammary tumour lines were also screened for the presence of casein mRNA sequences. Because of the previously reported sequence divergence of mouse and rat casein mRNAs¹³, a homologous cDNA probe was synthesised using a purified 15S mouse casein mRNA fraction as the template for avian myeloblastosis virus RNA-directed DNA polymerase. This cDNA probe displayed similar hybridisation kinetics with the purified mouse 15S casein mRNA and a total RNA preparation obtained from lactating mice as previously observed for the rat cDNA and mRNA fractions (Fig. 4). In addition, casein mRNA sequences were detected in RNA extracts obtained from 14-d pregnant mouse mammary tissue at levels less than one-fifth those observed during lactation, illustrating the sensitivity of the hybridisation assay. In none of the three autonomous mouse mammary tumour RNA samples tested, however, was any significant hybridisation detected. This assay is capable of detecting

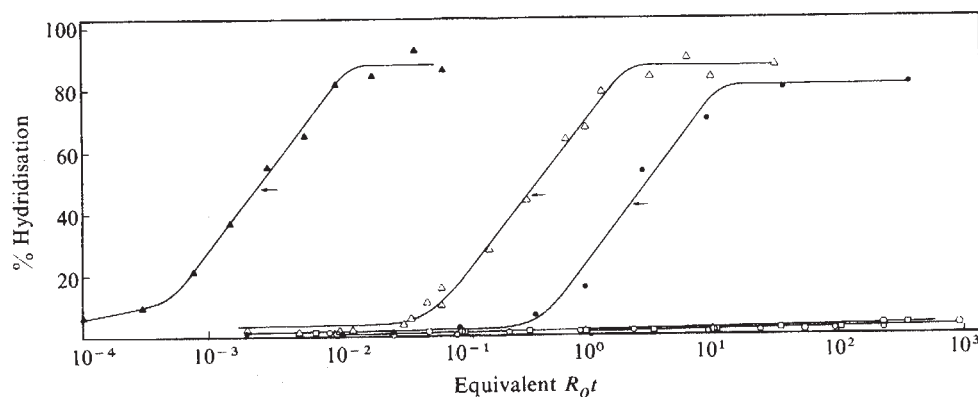


Fig. 4 Measurement of casein mRNA sequences in hormone-dependent mouse mammary tumours. The mouse mammary tumours used in these studies were transplantable lines originally induced by DMBA administration to BALB/c mice. These tumours grew equally well in intact female or ovariectomised animals. Hybridisation of mouse ³H-cDNA with purified mouse 15S casein mRNA (▲), RNA isolated from a 10-d lactating mouse (△), RNA isolated from a 14-d pregnant mouse (●) and RNA isolated from three different transplantable lines of mouse mammary carcinomas induced with DMBA (○, ◇, □).

casein mRNA sequences at levels of less than at least 0.1% of the normal lactating gland. Thus, these three tumours seem to represent variants, which have lost the hormonal responsiveness characteristic of the normal mammary gland and the accompanying expression of differentiated function.

The absence of casein mRNA sequences in the hormone-dependent mouse mammary carcinomas is consistent with the autonomous growth of these tumours. The failure to synthesise casein mRNA in response to endogenous hormones may reflect the absence or low levels of oestradiol and prolactin receptors in these cells¹¹. A more thorough examination of other tumour variants, especially receptor-positive cells which are unable to synthesise casein, may help elucidate the mechanisms regulating casein synthesis. A larger selection of both hormone-independent and dependent rat and mouse mammary tumours should be screened before a definitive correlation between the presence of casein mRNA sequences and hormone independence of mammary tumours can be ascertained. The detection of casein mRNA in hormone-dependent rat mammary carcinomas in this study, however, is consistent with the previously reported expression of normal, differentiated function in both experimental^{16,17} and human breast cancer¹⁸⁻²⁰. In addition, prolactin-inducible casein mRNA activity has also been detected recently in experimental breast cancer using a cell-free translation assay²¹. In conclusion, the data presented in this communication suggest that casein mRNA may be a useful molecular marker for determining hormone dependence, in addition to the presence of steroid and peptide hormone receptors.

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JEFFREY M. ROSEN
SUSAN H. SOCHER

Department of Cell Biology,
Baylor College of Medicine,
Houston, Texas 77030

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to the transcribing proteins. Transcription and translation then result in synthesis of proteins characteristic of the particular differentiated state. The interactions of metals and other ions with repetitive DNA (ref. 3) may be important in this recognition.

The model is further described in the following example where S_1 , S_2 and S_3 are structural genes specifying proteins P_1 , P_2 and P_3 and R_1 , R_2 and R_3 are repetitive sequences. Suppose that a sequence of DNA is

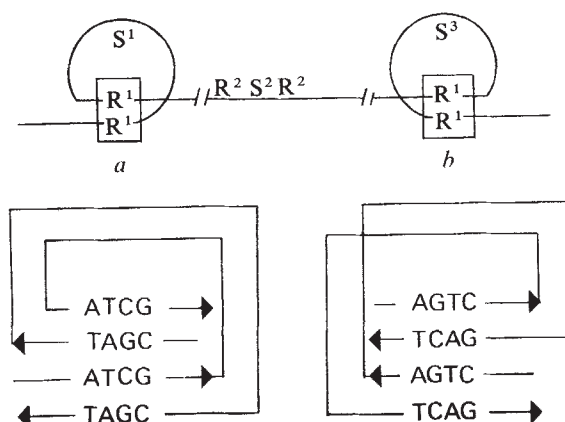
$$R_1 S_1 R_1 \dots R_2 S_2 R_2 \dots R_1 S_3 R_1$$

and that P_3 is a tissue-specific recognition protein that initiates and stabilises the association of double-stranded repetitive sequences R_1 with each other. These R_1 sequences need not be identical, but if they are identical they must be orientated in opposite directions (Fig. 1). This results in a localised distortion and/or unwinding of the DNA double helices (perhaps through the formation of a localised four-stranded structure) which is transmitted to the encompassed DNA, thus loosening the 'nucleosome' structure and making the DNA available for transcription. Thus both S_1 and S_3 , but not S_2 , are transcribed and translated giving proteins P_1 and P_3 characteristic of the particular tissue. The presence of protein P_3 will maintain the particular differentiated state. Such an arrangement of repetitive and unique DNA is consistent with the data (but not the analysis) of Davidson *et al.*⁴

Since most proteins are synthesised in more than one tissue, it is necessary to postulate that a structural gene may have more than one associated repetitive sequence. This will result in a continuum of repetition frequency classes, the frequency of each is dependent on the number of different cell types in which its associated structural gene is expressed. For example, $R_1 R_3 S_1 R_1 R_3 \dots R_2 R_3 S_2 R_2 R_3 \dots R_1 S_3 R_1$. Thus, in different tissues the genes expressed are: (a) S_1 and S_2 ; (b) S_2 ; (c) S_1 and S_3 . Such an arrangement of repetitive and unique sequences is suggested by Cech and Hearst⁵. Where several sequential genes are coordinately expressed in a given tissue, the group will be encompassed by the appropriate repetitive elements.

Development of the differentiated state can also be explained. For example, suppose cell a differentiates to give cell b which gives rise to the mature cell c. The genome of cell a is stabilised in its specific conformation by protein X and produces proteins A, B and C. At cell division X is broken down and nuclear reprogramming occurs⁶. Protein C thus has access to the genome and stabilises it in the conformation characteristic of cell b, which produces proteins D, E and F. At the next reprogramming, protein F has access to the genome and stabilises it in the conformation characteristic of cell c which produces proteins F, G and H. The continuing presence of F ensures the maintenance of the differentiated state.

Fig. 1 Interaction of tissue-specific recognition protein P_3 stabilises the association of double-repetitive sequences R_1 with each other. a, Identical R_1 sequences; b, non-identical R_1 sequences. As a result, structural genes S_1 and S_3 , but not S_2 , are made available for transcription.



A theory for genetic regulation by chromosome folding

THIS theory to explain gene expression in higher organisms is based on the spatial re-arrangement of genes by directed, specific folding of the chromosome. Tissue-specific differences in genome structure exist¹ and profound modulation of genome structure can occur during differentiation². It is postulated that specific proteins recognise specific sequences in double-stranded repetitive DNA and that, as a consequence, the structural genes encompassed by these repetitive sequences become accessible

Such transcription active regions may be equivalent to the interbands of dipteran polytene chromosomes⁷ and the 'loops' observed in lampbrush chromosomes⁸. Such structures are dynamic in that they show developmental and tissue-specific differences in size and configuration⁹.

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STEVEN VAUGHAN

Wadham College,
Oxford, UK

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Water binding by antifreeze glycoproteins from Antarctic fish

Low molecular weight glycoproteins isolated from serum of Antarctic fish have been shown¹ to be responsible for freezing point depression which permits survival at temperatures down to -1.9°C . The principal active antifreeze glycoprotein (AFGP) of several Antarctic species consists of a repeating tripeptide Ala-Thr-Ala with a disaccharide galactosyl-*N*-acetylgalactosamine linked to the threonine residue^{2–4}. The AFGP is more effective in freezing-point depression on a weight basis than NaCl and several hundredfold more effective than ordinary proteins². Melting point, however, is virtually unaffected by the AFGP⁵. We have investigated the possible role of protein-bound water in the unusual antifreeze activity of the AFGP. Ordinary proteins such as bovine serum albumin, haemoglobin, and lysozyme show hydration levels of about 0.4 g H₂O per g protein⁶. This water does not freeze at temperatures down to -35°C , but is sufficiently mobile to produce a measurable proton magnetic resonance signal. Quantitative determination of the amount of this 'bound' water is therefore possible in frozen protein solutions⁷. We have used this technique to examine water binding by the AFGP over a range of temperatures below the freezing point of bulk water.

Antifreeze glycoproteins 1–6 (ref. 2) were isolated from *Dissostichus mawsoni* (Norman) at McMurdo Sound Station, Antarctica and purified by DE-22 cellulose ion-exchange chromatography. The product was dialysed exhaustively against distilled water and lyophilised. Samples for magnetic resonance were prepared in 0.01 M KCl at concentrations of 25–100 mg ml⁻¹ determined by weight and ultraviolet absorbancy. Solutions of standard globular proteins, bovine serum albumin (Schwarz-Mann, crystalline, 100%) and human oxyhaemoglobin (gift from R. Banerjee) were prepared similarly. Nuclear magnetic resonance (NMR) spectra were recorded on a Perkin-Elmer R12 spectrometer operating at 60 MHz. No accumulation procedures were necessary. The samples in commercial NMR tubes were cooled from room temperature in 5° steps to -5°C . At this temperature solvent alone froze and the proton resonance signal disappeared completely. In the case of the protein solutions which supercooled, bulk water freezing was induced by lowering the instrument temperature control to -20°C until the first indication of loss of signal was observed (about 2 min). The control was returned to -5°C and signals were followed until equilibrium (constant peak area and line width) was achieved. The time dependency of the signal response was followed with each subsequent change of temperature in 5° increments to -40°C (cooling curve) and in the return to the melting temperature of the solution (warming curve). Peak area stabilised in about 15 min, line width

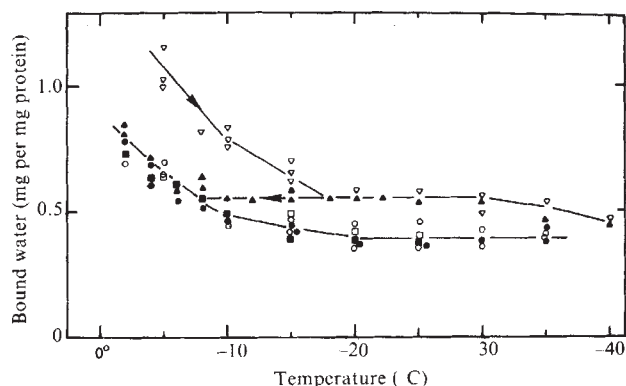


Fig. 1 Amounts of 'bound' (unfrozen) water in protein solutions at temperatures below the freezing point of bulk water. Open symbols represent data taken during cooling from -5°C ; closed symbols are data collected during warming. ∇ , Δ , AFGP; $\circ$, $\bullet$, bovine serum albumin; $\square$, $\blacksquare$, human haemoglobin.

in about 30 min, after a temperature change. The reported data were obtained after equilibration for 60 min; in these conditions line widths for cooling and warming coincided except in the case of the AFGP above -10°C . Line width values represent the width at half-height of the absorption curves. Areas were calculated as the product of line width and height and were converted to absolute values for hydration by comparison with a standard water sample (4.5 M LiCl, 0.01 M MnCl₂)⁷.

Figure 1 presents hydration results for the three proteins studied during cooling from -5 to -35 or -40°C and subsequent warming to -2°C . Amounts of unfrozen water were directly proportional to protein concentration in the range studied. In the case of the two globular proteins identical results were obtained during cooling and warming. At temperatures of -20 to -35°C the hydration averaged 0.40 g per g protein, in agreement with previous studies^{6–9}. In the AFGP, hydration at the low temperatures is 0.55 g per g protein; above -15°C hysteresis in the temperature dependency is observed. During cooling, hydration values as high as 1.1 g per g protein are observed, whereas with warming values of about 0.6 g per g are found, as in the two protein standards.

Line width data show the expected exponential increase with decreasing temperature (Fig. 2). Arrhenius activation energies are 4.0, 6.5 and 7.7 kcal mol⁻¹ for the AFGP, haemoglobin and serum albumin, respectively. The results for the two standard proteins are thus in good agreement with previous values for activation enthalpies of protein-bound water: 7.3 kcal mol⁻¹ for haemoglobin (from dielectric relaxation)¹⁰ and 5.0 kcal mol⁻¹ for BSA (from proton resonance in frozen solutions)⁹. The result obtained for the antifreeze glycoprotein falls within the range observed for polysaccharide hydration, 3.6–4.8 kcal mol⁻¹ (ref. 11). Estimated activation entropies^{8,9} in this study are -9 e.u. for AFGP, $+4$ e.u. for haemoglobin, and $+9$ e.u. for bovine serum albumin. Comparison of results for the three proteins suggests that the higher level of hydration in the AFGP is associated with somewhat weaker binding, presumably fewer hydrogen bonds, and a less structured environment.

Relaxation behaviour in the frozen AFGP solutions during cooling was suggestive of a two-phase system with slow exchange¹². Thus, at -5°C a sharp resonance of line width about 100 Hz seemed to be superimposed on a broad band of width 600 Hz (Fig. 3), merging at lower temperatures. Both were distinguishable from bulk water which, before its freezing by means of the cold pulse, showed a line width less than 10 Hz. The additional component of unfrozen water in the AFGP apparently represents a category intermediate between Type I (bulk

water) and Type II (bound water)⁹. In quantitative terms the 100 mg ml⁻¹ AFGP solution at -5 °C contains about 90% Type I water, 5% Type II water, and 5% additional unfrozen water. Type III, irrotationally bound water⁹, is not measurable by this technique, but is anticipated to be less than 0.1%.

The level of Type II water in the AFGP seems to be consistent with its chemical composition. On the basis of hydration values of 1.5 H₂O/alanine residue and 2 H₂O/threonine residue¹³, hydration of 0.37 g per g protein would be predicted for the copolymer (Ala-Ala-Thr)_n. Since the polypeptide accounts for 39% of AFGP weight², it would therefore contribute 0.15 g H₂O per g AFGP to the total hydration. Hydration of the sugar portion of the molecule would have to be 0.66 g per g carbohydrate to account for the remaining AFGP hydration. This figure is similar to the value of 0.59 g per g reported for Agarose¹¹. The magnitude of the line width for the Type II water in the AFGP is consistent with an unstructured conformation of the molecule, as shown by circular dichroism² and confirmed for the samples used in this study. Signal broadening has been observed in urea-denatured bovine serum albumin⁶ and in random-coil polypeptides⁹.

Of particular interest is the possible significance of either the Type II water or the additional unfrozen water in the AFGP relative to freezing-point depression. AFGP occurs as several molecular forms in fish plasma at a total concentration of about 25 mg ml⁻¹; the active forms studied here account for up to 10 mg ml⁻¹ (ref. 2). Thus, the amount of water bound by the AFGP in plasma represents only 1% of total water. Nevertheless, the similarity in temperature-dependent hysteresis of the resonance data and that of the freezing-melting behaviour of the AFGP¹⁴ suggests a possible role for this water in the antifreeze activity. Studies of partition of the AFGP in frozen solutions indicate that the AFGP molecules are themselves incorporated into ice during freezing¹⁵,

Fig. 2 Arrhenius plots of line width data for unfrozen water in protein solutions. ▽, ▲, AFGP; ○, ●, bovine serum albumin; □, ■, human haemoglobin.

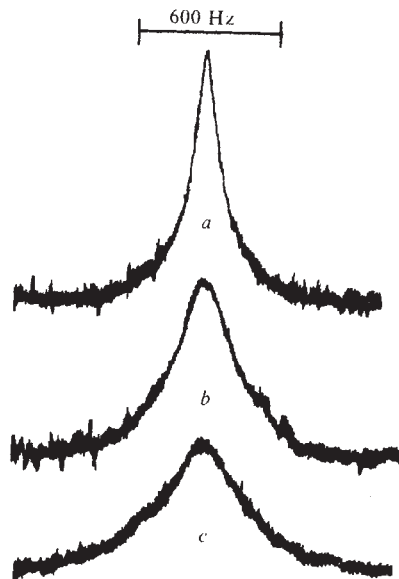
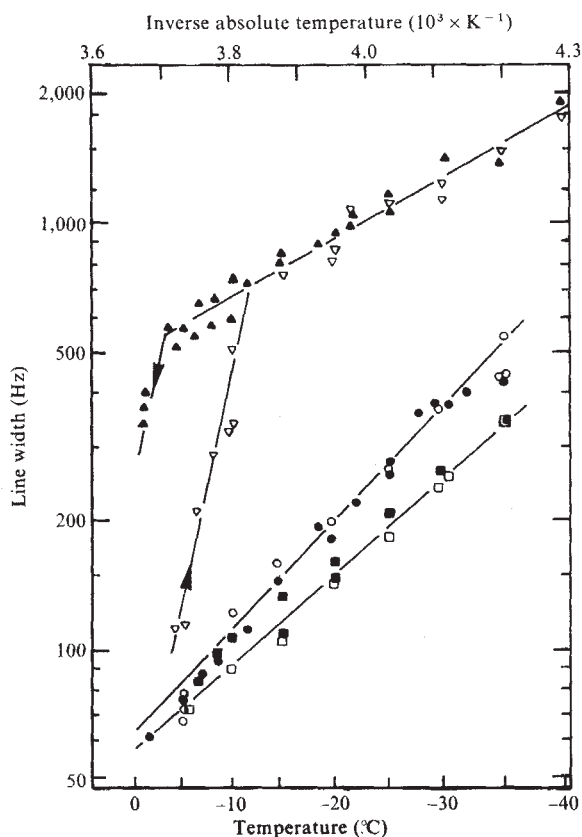


Fig. 3 Proton resonance spectra of unfrozen water in solutions of the antifreeze glycoprotein at -5 °C (a), -8 °C (b) and -10 °C (c).

although they do not affect other freezing characteristics such as salt compartmentalisation¹⁶. It has been suggested that AFGP may prevent ice propagation by direct binding to ice surfaces, thus increasing surface free energy or simply coating the ice with a barrier of unfreezable water¹⁷. Normal protein-bound Type II water is apparently not effective in freezing-point depression, as evidenced by the absence of this activity in ordinary proteins². The explanation for AFGP activity, however, may lie in the elevated levels of water that is resistant to freezing, and in the extended conformation of the molecule which serves to optimise surface contacts with ice crystals. Comparative studies of water binding in model polysaccharides and in related fish glycoproteins which lack antifreeze activity should shed further light on the activity of this unusual protein.

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A. E. V. HASCHEMEYER*
W. GUSCHLBAUER

Service de Biochimie,
Centre d'Etudes Nucleaires de Saclay,
B. P. No. 2, F-91190-Gif-sur-Yvette, France

ARTHUR L. DEVRIES

Department of Physiology and Biophysics,
University of Illinois,
Urbana, Illinois 61801

Received 23 March; accepted 14 July 1977.

*Permanent address: Department of Biological Sciences, Hunter College, City University of New York, 695 Park Avenue, New York 14208.

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reviews

The problematique

Eric Ashby

The Human Quality. By Aurelio Peccei. Pp. 214. (Pergamon: Oxford and New York, 1977.) Paperback, £3.25; \$6. Hardback, £6.50; \$12.

To write about this book as a reviewer is easy, but as a critic is difficult. I shall try to do both.

The early part of the book is an artless life-story of a modest, sincere, and courageous man. Aurelio Peccei was a freedom-fighter in the Resistance in Italy, imprisoned at one time by the Nazis. He then became a highly successful industrialist. It is ironic, in the light of his present activities, that his industrial success was to provide large numbers of mass-produced automobiles which polluted Europe and—through Peccei's special efforts—Latin America.

But Peccei's conscience survived this material success and he turned in 1957 to something with a dash of altruism added to industrial acumen: an engineering and economic consultancy which was to do work in developing countries on a non-profit-making basis. It was not a Pauline conversion exactly, for Peccei still maintained his connections with the manufacture of automobiles, but he had come to the view that multinational companies have a responsibility to society above that of making profits for their shareholders. By the 1960s, his family being now grown up and his level of affluence being adequate, Peccei found himself free to reflect on human destiny.

The rest of the book describes Peccei's reflections and the practical steps he has taken to disseminate them. The most significant of these steps was the formation of the Club of Rome, which is an informal group of people who are concerned with what they call the *problematique*. The *problematique* is (Peccei writes) the "intractable tangle of difficulties" which constitute the present "predicament of mankind."

That there are intractable difficulties facing industrial societies and the developing nations no-one questions for a moment. But that Peccei and the Club of Rome are the first people to have realised this, is nonsense. The Club (writes Peccei, observing that "we know incredibly little about our

changed conditions") is "the first to rebel against this well nigh suicidal ignorance." This claim to priority is just one of a string of unacceptable assertions in the book. Has the Club of Rome not heard of H. G. Wells, Aldous Huxley, George Orwell, Lewis Mumford, Bertrand de Jouvenal? Were they not aware of the *problematique*?

Peccei offers a somewhat querulous defence of the best-known project of the Club of Rome, which was to sponsor the book *Limits to Growth*. The warning in this book, that continued exponential growth would lead to collapse, was (Peccei writes) "denounced as nothing more than a heretical and misinformed libel." This is a grossly inaccurate way to describe the sober and careful criticism which *Limits to Growth* received. Peccei does nothing to refute these criticisms; indeed he does not even mention what the criticisms are: not a word about the reasoned indictments from the University of Sussex or the World Bank. These critics were not (as Peccei suggests) defending the "sacred cow of growth" nor were they under the impression (as Peccei suggests) that the Club of Rome advocated zero growth. The critics were simply exposing the spurious assumptions which had been fed into MIT's computer. In a later chapter, but without acknowledging the debt which the Club of Rome owes to these critics, Peccei concedes some of their criticisms. "Perhaps," he writes, "science and technology are capable of placing at mankind's disposal the immense resources required. . . ."

It is over 50 years since Raymond Pearl published data on the sigmoid curves of population growth and discussed some of their consequences. The Club of Rome has performed a useful service in stimulating popular interest in the climacteric of modern society: the transition from accelerating growth rate with positive feedback to decelerating growth rate with negative feedback; and I am willing to acknowledge that the issue had to be made sensational in order to capture public attention. But the basic strategic error which the Club has made (and which Peccei makes even more egregiously) is to deflect people from paying attention to thousands of mini-

solutions to mini-problems (which is the way society is most likely to survive this transition) by dangling before them one Comprehensive Maxi-Solution.

Assertions about the maxi-solution occupy most of the space in Peccei's book. They are offered in the tedious platitudes which are the despair of people who are seriously engaged in the minute particulars of trying to improve the condition of society. "[S]ociety must see to it that *all* the benefits which the system can provide—including goods and services—are actually put at the disposal of *all*." Mankind "must undergo" a process of "total re-education." It is the use of the word "must" which riles me. Even if you accept the premise—that "must" implies "ought"—there are two consequential questions which follow the use of the word "must"; one is: "How?" The other is: "Imposed by whom?" Neither Peccei nor the Club of Rome offer any answers to these two questions. Without answers, these intoxicating declarations simply save people the trouble of considering far more important problems, such as how to see to it that *some* of the benefits of the system can be put at the disposal of *some* people, and that in place of the airy policy of total re-education for *everyone* (New York stockbrokers, Italian politicians, Indian peasants, Ghanaian cocoa farmers, Moscow policemen!), there is encouragement for thousands of people who are trying to do a little re-education for a few.

So I reject Peccei's whole approach to the predicament of mankind on the simple criterion that these vague and sweeping clichés which he offers as the recipe for achieving social justice are as impotent to solve the *problematique* as similar vague generalisations would be for the production and sale of automobiles. But—and here I am in complete agreement with Peccei—the *problematique* (much as I dislike the word) does stand for something real which confronts governments all over the world. What can take the place of Peccei's sincere but futile gestures to mankind at large? I think there are modest and much less colourful alternatives. It would be more effective if diagnoses of the predicament of mankind were to separate

clearly what *is* (that is, to get the facts straight) from what *ought to be* (which is legitimate ethically provided you say *why* it ought to be); and never to proceed to "*must*", for on that way lies compulsion, dictatorship, the rule of Leviathan. A wise assumption would be that social attitudes cannot be changed except incrementally, and that there are "tolerance limits", so to speak, to the magnitude of a change which people will contemplate and be prepared to accept. Any proposal which lies outside these tolerance limits is rejected; it creates resentment, sales resistance, is (in modern jargon) counter-productive.

And, finally, all attempts to define goals for mankind are likely to be mirages, vanishing as one approaches

them. There is another way to move toward goals: that is not to demand a grandiloquent metamorphosis of mankind, but instead to encourage little groups of people all over the place to make wise choices over small changes in society. For it is one lesson of history (and it would do the Club of Rome no harm to read a little history) that ends are continually being re-defined by the means being used in groping toward those ends; and it is another lesson of history that *men and women* do respond to messages which lie within their range of understanding, but *mankind* is deaf to all messages. □

Eric Ashby is at present Walgreen Professor of Human Understanding at the University of Michigan.

GC-MS system

Fundamentals of Integrated GC-MS. Part III: The Integrated GC-MS Analytical System. By B. J. Gudwinowicz, M. J. Gudwinowicz and H. F. Martin. Pp. vii+603. (Dekker: New York and Basel, 1977.) SFr.190.

This third part of the text on analytical chemistry was written in mid-1976 and surveys knowledge about the integrated gas chromatography-mass spectrometry (GC-MS) system up to that date.

The first chapter deals with basic vacuum technology and the interface between the GC and MS, seeks to provide enough of a mathematical treatment of gas flow characteristics to permit an understanding of the problems associated with interfaces to be appreciated. All of the usual enrichment devices are discussed in detail and their characteristics compared. The authors have made excellent and very full use of tables, graphs and drawings of apparatus as well as including in the text many references to original work. The chapter contains 181 pages with 18 tables, 88 figures, 263 mathematical equations and 221 references.

The middle chapter deals with the methods which are available to optimise the operational parameters of each separate part of the integrated GC-MS analytical system. The authors consider each operational parameter in turn and deal with the optimisation of carrier gas flow rates, injection systems, the thermostability of liquid phases, chromatography columns of various types, inlet and effluent splitters, separator enrichment factors and characteristics, source temperature and pressure, ionising potentials, sensitivity, scan rates, dynamic resolution, ratio recording, and the use of peak matching techniques to measure the

accurate mass of fragment ions during GC-MS scans. This long chapter is fully illustrated and well referenced, containing 202 pages with 29 tables, 96 figures and 484 references.

The final chapter deals with the role of a mass spectrometer as a GC detector and the data processing of the mass spectral results. The role of the ion monitor in producing a total ion monitor (TIM) graph is discussed, and multiple ion monitoring and single ion monitoring techniques are reviewed in detail. Several examples of the use of mass fragmentography techniques for the analysis of biological samples are described and the sensitivity of detection and quantification using single ion monitoring are discussed.

The history of the application of data processing techniques to mass spectrometers is reviewed and the use of these systems for the calculation of the accurate masses and the molecular formulae of fragment ions, and their display in terms of element maps, is described. Repetitive cyclical scanning GC-MS-COMP systems are described where the mass and abundance information is stored in disc systems ready for regeneration in the form of individual mass spectra, outputs from library search routines or mass chromatograms. The final chapter contains 140 pages with 15 tables, 68 figures and 171 references.

The authors have provided a book which is a mine of information for those involved in, or about to embark on this field. It is prepared to a high standard and is well indexed. It is a 'must' for every GC-MS laboratory but it is priced (190Fr=£46) well beyond the resources of most individuals.

James A. Ballantine

James A. Ballantine is Senior Lecturer and operator of a GC-MS system in the Chemistry Department of the University College of Swansea, UK.

Statistical mechanics revised

Statistical Mechanics. Second edition. By J. E. Mayer and M. G. Meyer. Pp. 491. (Wiley: Sussex, New York, Sydney, Tokyo, and Mexico City, 1977.) £17.50; \$29.

A CLASSIC BOOK revised by its author 37 years later for a second edition has to be approached with the respect one would normally reserve for, say, a fine old whisky. This respect is indeed deserved, for this book has retained its clarity, relevance and coverage. It has nevertheless lost some of the quality of the first edition: the small print—an author's whisperings into a reader's ear—has gone. So has the author index; so have the problems and the glossary. The effect is that the material, though necessarily altered, covers the same number of pages as the first edition. I bought my 1948 copy, however, for one-seventh of the present price.

Turning to the contents, substantial parts of the book are unchanged; for example, the early chapters and also the treatment of electric and magnetic fields. The discussion of black-body radiation is postponed to the quantum theoretical part, as it was in the first edition, and so comes rather late (p368) for so important a topic. On the other hand, new sections on computer experiments (p320), on the coupling of nuclear spins to electrons and on spin echo, up-date the book in the author's lively style. Some unexpected references are left, for example (p38), one to Gibson's Amsterdam thesis of 1933, which readers are unlikely to want to look up to check on the viscosity values for hydrogen. A "constant entropy paradox" is named, stated, and resolved, but the simple lesson that coarse-graining is to some extent responsible for entropy increase is not very explicitly drawn. There is not much on stability or negative temperatures and on p106 the author might have noted that heat capacities *can* be negative (in gravitational systems).

This book, written by two great experts in the field, and now revised with extreme care by one of them, remains a good introduction, is a source of much useful information, and should enjoy continued popularity.

Peter Landsberg

Peter Landsberg is Professor of Applied Mathematics at the University of Southampton, UK.

Heterocyclic compounds

Stereochemistry of Heterocyclic Compounds. Part 1: Nitrogen Heterocycles. By W. L. F. Armarego. Pp. 443. Wiley: London, New York and Sydney, 1977. £27; \$45.

DR ARMAREGO'S aim is to survey the entire stereochemistry of heterocyclic compounds, excluding most natural products, in two volumes. He has chosen to do this by referring freely to specialised reviews and other secondary sources, thereby avoiding duplication of effort and leaving himself free to give quite detailed discussion to the remainder of the subject, with emphasis on recent work. This approach clearly adds to the value of the book to the specialist but runs the risk of bittiness and lack of balance for the more general reader. Fortunately, Dr Armarego's style and wide-ranging interest in his subject save the book from these dangers, and large parts can be read with enjoyment and a sense of continuity.

This volume is concerned with compounds with nitrogen as the only heteroatoms in the rings and is divided into a short introductory chapter and

three very long chapters with compounds classified by ring size: chapter 2 covers three-, four-, and five-membered rings, chapter 3 six-membered rings, and chapter 4 the larger rings. Each chapter is subdivided into increasingly complex classes of compounds in a way that makes it generally easy to locate information without working through the detailed table of contents.

A book of this type must be up to date, reliable and uniform in its coverage of the original literature, clear in its diagrams, and concordant with currently accepted theory in its interpretations of the properties of the compounds described. In most respects Dr Armarego's book meets these requirements well. The references are very full for the period from 1965 to about the middle of 1975. A check on the references in chapter 3 showed that the average number each year for 1965 onwards is about six times as high as for 1964 and earlier. The abrupt change in the frequency of references suggests the possibility that Dr Armarego's book may have gaps in respect to the earlier literature but I have not found any that are important and not indirectly covered by the references to secondary sources. Occasionally there is a lack of balance: for example, Kipping's work on 2,3,5,6-

tetramethylpiperazine in the 1930s is described in some detail when a reference to Harris and Sheppard's conclusive NMR study in 1955 would have been sufficient. In contrast, the diazabicyclo[3.3.1]nonanes are dismissed very briefly. This section contains a rare but bad example of chemical misunderstanding because the 'normal' amide carbonyl group in 3-isopropyl-2-oxo-1,3-diazabicyclo[3.3.1] nonane is regarded as a violation of Bredt's Rule and is inappropriately contrasted with the ketonic carbonyl group in 2-oxo-1-azabicyclo[2.2.2]octane. The formulae are usually clear and well drawn, particularly when this is most important as with bridged and caged polycyclic systems, but there are, unfortunately, some very distorted drawings of fused six-membered rings (notably, formulae 260 and 296 in chapter 3) that result from the uncritical use of stencils.

Dr Armarego has been very successful in realising his aim. This book will be bought by many chemists with interests in stereochemistry or heterocyclic chemistry, as well as by all chemical libraries. **M. J. T. Robinson**

M. J. T. Robinson is a University Lecturer in Organic Chemistry at the University of Oxford and a Fellow of Magdalen College, Oxford, UK.

Weak interactions

Weak Interactions. By David Bailin. Pp. ix+406. (Chatto and Windus: London, 1977.) Paperback £9.

THE subject of weak interactions has developed very rapidly in the past decade. As a result there is a fairly wide and growing gap between good old textbooks, such as the well known book by Okun, and the moving frontiers. Since there are no signs that the motion of the frontier is slowing down, it is wise to educate graduate students in particle physics in the field of weak interactions. David Bailin was aware of this real problem and this book is his answer to it.

The book starts with a lengthy introduction to the Dirac equation and the construction of invariant amplitudes for fermions, and then discusses in detail leptonic, semileptonic and non-leptonic weak interactions as well as CP violation and unified gauge theories.

The new knowledge gained in the past decade is indeed covered by Bailin's book, which is useful as a guide to what is known and as an introduction to the literature. Its main strength lies in the material which is covered and the detailed elaboration

of computational details. I could find very few misprints and no errors of calculation.

The emphasis on calculations is also the source of the main weakness of the book: it is lacking in pedagogy. There is much more material than could be covered in a one-year course. The author could, however, have avoided stressing hard computation rather than easy insight. Many topics are discussed in a way which, though correct, are likely to be difficult for beginners. For example, SU_3 and the Cabibbo angle are introduced in an abstract fashion starting with the structure constants f_{ijk} of the algebra. It is much more easy to start with a quark model and introduce the Cabibbo rotation at the level of the quark currents and only talk about SU_3 afterwards. This way is also easier to generalise to a larger number of quarks—a contemporary problem.

I recommend Bailin's book to all those who want to become familiar with modern developments in theoretical technology for weak interactions. The publishers must anticipate a huge increase in graduate student grants, judging from the price of this book.

G. Karl

G. Karl is Professor of Physics at the University of Guelph, Ontario, and is at present on sabbatical leave at the SRC Rutherford Laboratory, Chilton, UK.

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Vector analysis

Vector Fields: Vector Analysis Developed Through Its Applications to Engineering and Physics. By J. A. Shercliff. Pp. 329. (Cambridge University: Cambridge and London, 1977.) Hardback £13.50, paperback £4.25.

THIS book is stimulating and occasionally irritating; but few stimulants are without their side-effects. It is aimed at physicists and engineers at the equivalent of British second-year undergraduate level. Teachers of engineering may be happier with it than teachers of physics, although many physics students might find that it taught them more than would a more conventional approach. The approach is unconventional, to this physicist at least, because it fuses the entire content of a classical electromagnetic theory course and of a hydrodynamics course in order to illustrate the tools and methods of vector analysis.

The introductory chapters certainly would not replace proper courses on basic electricity and magnetism or on vectors. The summary is brief and might be rather obscure to, for instance, a student whose knowledge of vectors was based on 'modern mathematics'. The argument is pictorial and intuitive, with some stress placed on a rather woolly property which all authentic vectors are supposed to have; 'cosine quality'. Someone who has played with scalar products, work integrals and components of forces will grasp 'cosine quality' straight away, just as someone who knows Fleming's left-hand motor rule will understand how magnetism is 'electricity looked at sideways'. The good thing about this cavalier treatment is that it should give the student a feeling for the eventual simplicity of material which he may have found heavy-going in the first year.

The next chapter is on line and surface integrals, assuming some knowledge of partial differentiation and of Taylor's theorem. Ampere's law, the Biot-Savart law and various aspects of current electromagnetism are followed by volume integrals, with illustrations from simple plasma theory. Then comes 'A crisis in electromagnetic theory', a rather wordy treatment of the arguments leading to the concept of displacement current. The stage now seems to be set for the derivation of Maxwell's equations. But no; this consummation is delayed for another five chapters. Divergence and gradient must be introduced; then Gauss' theorem, Laplace's equation, a variety of applications to hydrostatics, fluid flow and heat flow, Poisson's and Helmholtz' equations with examples including the

criticality condition in a nuclear reactor. The treatment of waves and of the superposition principle is surprisingly sketchy after the detail given to many other topics.

Curl comes next, with a great deal of illustrative detail in hydrodynamics, leading to Stokes' theorem. Then, at last, we are ready for Maxwell's equations, followed by discussions of the skin effect, electromagnetic waves and the Poynting flux. The final chapter is on 'Integrals in fluid mechanics', beginning with derivations of Kelvin's and Helmholtz' theorems and applying them to such problems as the jet from an orifice, flow over an infinite airfoil and the lift and drag properties of an aircraft wing.

The book is illustrated with a large number of clear, simple line drawings.

There are more than two hundred problems on a great variety of applications, distributed in clumps in mid-chapter as well as at the ends of chapters. Answers are provided to all the problems.

Although most departments will not wish to telescope courses together in the way Shercliff has done, the book should be generally useful as an additional text. Weak students will benefit from the wealth of applications for each abstract idea. Stronger students may be led on to read and understand material which a more formal textbook would leave out.

David J. Miller

David J. Miller is Lecturer in the Department of Physics and Astronomy at University College, London, UK.

Search for life

Chemical Evolution of the Giant Planets. Edited by Cyril Ponnampereuma. Pp. 240. (Academic: New York and London, 1976.) \$11.50; £8.15.

THIS book contains the invited lectures presented at the first of a series of colloquia to be organised by the Laboratory of Chemical Evolution at the University of Maryland, aimed at bringing together scientists for formal and informal discussion on topics pertinent to chemical evolution. The meeting, held in October 1974, was stimulated by the availability of data from NASA's Pioneer 10 and 11 and attended by astronomers, chemists, biologists and engineers, involved with, or interested in, the outcome of those missions. The overall theme of the discussion was intended to consider whether conditions prevail in the atmospheres of the giant planets which are conducive to chemical synthesis, or able to support life of a terrestrial nature. The title of the volume may be to some extent a misnomer since many of the authors did not restrict themselves to the location suggested. The outer planets in general, Mercury, Venus, Mars, meteorites, comets and the satellites of Jupiter and Saturn, particularly Titan, are freely discussed.

The book consists of sixteen papers by twenty authors; its content may be broken down into several general subject areas. T. Gehrels, D. M. Hunten and T. Owen provide essential background information concerning atmospheric composition, structure and chemical composition. W. F. Libby, J. S. Lewis and C. Ponnampereuma cover various aspects of chemical synthesis in the Miller-Urey vein. The biological

papers (R. D. MacElroy, D. J. Kushner, R. L. Dimmick and M. R. Chatigny, R. S. Hanson and N. H. Horowitz) centre on the adaptability of microorganisms and their ability to survive in harsh environments having a variety of temperatures, pH, salt content, and even in aerosols. The energy requirements of a biosphere in terms of light input are considered separately by B. Kok and R. Radmer. Two essentially technique papers by E. E. Russel and M. G. Tomasko, and R. A. Hanel cover spin-scan imaging and infrared spectroscopy, respectively. NASA philosophy towards further planetary exploration is put forward by D. H. Herman and S. J. Grivas. Because of funding constraints rather than through any fault of the authors, the treatment is of a very general nature. A more important philosophical question raised by R. S. Young and R. D. MacElroy, concerns policy with respect to the possible irreversible contamination of any planetary surface or atmosphere, by organisms or molecules, carried on unsterilised or uncleaned space probes.

All in all, I feel this is a very useful book. Not, perhaps, of great value to the specialist chemist or biologist, but eminently readable by anyone with fringe knowledge of a variety of subjects and interested in studies on the origin of life. It provides many appropriate references and has a full index. As the object of the colloquium was to provide a forum for discussion, I wish an edited version of the question and answer sessions could have been included at the end of each chapter.

C. T. Pillinger

C. T. Pillinger, of the Department of Mineralogy and Petrology, University of Cambridge, UK, is a principal investigator in NASA's lunar sample analysis program.

obituary

Benjamin Lee

PROFESSOR BENJAMIN W. LEE died in an automobile accident on 16 June 1977. Director of Theoretical Physics at Fermi National Accelerator Laboratory and Professor of Physics at the University of Chicago Enrico Fermi Institute, Ben Lee was at the age of 42 deeply engaged in scientific activity at the forefront of high energy physics.

Born in Seoul, Korea, Ben Lee emigrated to the United States in 1956 and obtained citizenship in 1968. He studied at Miami University (Oxford, Ohio), the University of Pittsburgh and the University of Pennsylvania, where he was awarded a doctorate in 1960. His earliest work dealt primarily with dispersion relations and related techniques in the analysis of strong interaction phenomena, but he also had an early interest in Lagrangian symmetries and spontaneous symmetry breakdown, which ultimately became the focal points of his research.

By 1964 Ben Lee was a leading contributor to the study of the transformation properties of weak interactions with respect to strong interaction symmetries, and the intimately related question of the full symmetry properties of the strong interactions themselves. Through such studies the relevance of a spontaneously broken chiral symmetry gradually became apparent, and Lee contributed significantly to the construction and study of Lagrangian field theories which realise this property. His work demonstrating the renormalisability of these theories provided an encouraging model for later developments which led in 1971 to the establishment of renormalisability of spontaneously broken gauge theories. Following this breakthrough, Lee's work on the formal demonstration of renormalisability of gauge theories and on the elaboration of calculation techniques was of major importance in the further development of this field which has taken a dominant position in our present thinking.

As soon as the relevance of gauge theories for weak interactions began to appear highly probable, Ben Lee turned to the study of their practical implications, both for the newly discovered neutral current interactions and for the previously established properties of charged current interactions.

The use of new theoretical developments allowed a confrontation with experiment which placed severe restrictions on the possible choice of gauge models. It became increasingly clear that a viable model should incorporate charmed particles which had been postulated before achievements in renormalisation made such an inference compelling. Lee devoted considerable study to the expected properties of these particles and the possibilities for their experimental detection.

Along with the experimental discovery of charm, there has been a continuous experimental and theoretical evolution in the field of both weak and strong gauge theories, in which Ben Lee continued to participate fully. His rare combination of a familiarity with formal mathematical techniques and a close touch with experimenters and experimental results kept him at the forefront of many different aspects of these developments. At the same time he continued to reflect on the outstanding problems related to his earlier work on weak interaction symmetries and was quick to apply new developments to the clarification of old problems. He encouraged precision experiments designed to resolve long-standing questions as well as pioneering experimentation on the new phenomena.

Ben Lee's decision to remain permanently as head of the Fermilab theory group was not an easy one for him. He felt that at a university he would be freer to pursue fundamental theoretical questions. Yet while continuing to participate in the fast pace of theoretical research, he took extremely seriously the responsibilities of his position at Fermilab. On the one hand he attracted theoretical physicists of the highest quality as visitors to the laboratory and was in the process of putting together a small but strong nucleus of resident theorists. On the other hand he was deeply involved in laboratory policy concerning its present and future experimental programme, a job which he undertook with singular conscientiousness, endeavouring to learn the fine points of experimental techniques.

Before joining the Fermilab staff in 1973, Benjamin Lee was Professor of Physics at the State University of New York at Stony Brook. In addition to his responsibilities at Fermilab he served on the scientific policy com-

mittees for several major laboratories. He was the recipient of a number of prestigious scientific fellowships and had held appointments at many institutions in the United States and abroad. Perhaps his most meaningful experience in recent years was a visit to his native Korea where he was struck by the developing prospects for scientific research and found himself "working six days a week on upgrading graduate education in basic sciences" at the Seoul National University.

Ben Lee shared a close family life with his wife Marianne and teen-aged son and daughter at their home in Glen Ellyn, Illinois. Whenever possible they combined business with pleasure on trips to Aspen in Colorado, Europe and the Far East. An immeasurable personal tragedy for his family, Benjamin Lee's untimely death is a deep loss for the entire high energy physics community.

Mary K. Gaillard

Fred Hardy

PROFESSOR FRED HARDY, CBE, who died at St Augustine, Trinidad, in May at the age of 88 was the doyen of British tropical soil scientists. During his professional life the study of the soil developed from a subject that had little impact on practical agriculture to a discipline that has made major contributions to agricultural development, and Professor Hardy played a notable role in this transformation for tropical soil studies.

Almost his whole adult life was spent in the West Indies and Central America. His first appointment after leaving Cambridge University, was as lecturer in natural and agricultural sciences at Harrison College, Barbados, in 1911. He returned to England in 1917 towards the end of the First World War, then after the war spent a year at the School of Agriculture at Cambridge and then went back in 1920 to the West Indies as soil chemist with the Imperial Department of Agriculture, which was centred at St Augustine. This later became the Imperial College of Tropical Agriculture, and he became its first Professor of Chemistry and Soil Science. After his retirement in 1956 he served as Professor of Soil Science in the Inter-American Institute of Agricultural Sciences at Turrialba, Costa

Rica, for nine years before finally retiring to Trinidad.

Hardy's research career began after his return to the West Indies in 1920. He began by being interested in problems of soil water and the effect of water on some soil properties; then in the 1930s he became interested in the characteristics and genesis of tropical soils and laterites; and finally in the 1940s and 1950s he developed his well known studies on the soil factors that affect the fertility of tropical soils and their suitability for specific crops. In addition he was one of the early British soil scientists who realised the need for appropriate soil surveys for agricultural development, and I believe the first in the colonial Empire to institute a systematic soil survey, and he succeeded obtaining funds over a period of years for a survey of most of the British West Indies.

Professor Hardy had, however, a far greater influence on British colonial agriculture than would be judged from his research work, for almost every recruit to the Colonial Agricultural Service since the mid-1920s spent a year in Trinidad and most would study soils under him. His enthusiasm, his great friendliness and approachability, and his gift for picking out the most important points in any discussion, all left their mark; and his appreciation of the relation of the soil and the crop to the natural landscape must have influenced the ecological approach to agricultural development shown by many outstanding colonial agricultural officers.

E. W. Russell

William Parker

THE sudden and untimely death of William Parker, Professor of Chemistry at the University of Stirling, at his home in Dunblane on May 9 at the age of 45, has left the world-wide chemistry community with an immense feeling of loss.

Born in Glasgow in 1932, Willie Parker was a distinguished pupil of Whitehill Secondary School after which he went to the University of Glasgow where he took a first class honours degree in Chemistry in 1953. He started his PhD studies in Glasgow with Professor R. A. Raphael and completed them at the Queen's University of Belfast when Raphael moved there to his first professorial post. In 1956 Willie joined Professor G. Stork's group at Columbia University and then returned to the University of Glasgow where he rapidly moved up the academic ladder, culminating in his appointment to a senior lectureship in 1966. Promotion to readership saw him back in Ireland in 1968 at the

New University of Ulster where he spent two happy years helping to build up the new chemistry department there with characteristic drive and enthusiasm under the leadership of Professor M. Grundon. Armed with this valuable experience he returned to Scotland in 1970 to take up the first chair in organic chemistry at the University of Stirling.

Natural products, particularly the sesquiterpenes, were Willie's first love and in this area he published many important papers relating to their synthesis, biogenesis and rearrangements. In particular, he and his co-workers unravelled many of the problems associated with the molecular acrobatics of caryophyllene and humulene and their derivatives. Although he maintained this interest throughout his research career, a sabbatical year with Professor J. Berson at the University of Wisconsin in 1965 served to promote and nurture his growing interest in organic reaction mechanisms with particular emphasis on the solvolytic behaviour and preferred conformation of a wide range of bridged bicyclic compounds. Of particular note were his contributions to the understanding of the reactivity of bicyclo[3,3,1]nonane, bicyclo[3,3,2]decane and bicyclo[3,3,3]undecane (manxane) derivatives. At the other end of the spectrum he also had a keen research interest in the chemical factors influencing germination and dormancy in wild oat seeds. Shortly before his death, his group, together with collaborators in other disciplines, had made significant advances in this area.

Over the past eighteen years I knew him in many different rôles; as a teacher, as a research supervisor, as a colleague, as an opponent on the squash court and, above all, as a friend. In all these aspects his boundless enthusiasm, inexhaustible drive, unadulterated Glasgow humour, and extrovert personality made a tremendous impact on me as they did on so many other people. If ever there was a teacher who practised the subtle art of instilling a sense of dedication and professionalism in his students there was no greater practitioner than Willie Parker. Under his guidance and tuition organic chemistry came across as an exciting and challenging field of study.

Over the years many students, of whom I was one, continued their studies with Willie as their PhD supervisor. He shared in our triumphs and disappointments at the bench, our laughter and our tears. All of us greatly benefited from that experience and I have no doubt that his guiding principles and aspirations had a profound influence on us.

His rumbustious character came

through vividly in all the many facets of his work at the University. Willie was by no means an isolationist in an ivory tower; he enjoyed all his teaching commitments whether in the first year undergraduate laboratory with his sleeves rolled up or at the Monday evening postgraduate seminars. He approached his administrative duties both in the chemistry department and in the many university committees on which he served with a refreshing, down-to-earth attitude.

More than anything else he wanted to and did put the Chemistry Department of Stirling University on the map. I believe that he achieved this not only by his own personal reputation in the world of organic chemistry and by his service on national committees such as the Chemistry Panel of the Science Research Council, but also by his active encouragement of other members of staff to apply for grants and to seek and establish links with the chemical industry. Above all he had the unique ability to muster and channel our individual energies towards the common cause of creating a resourceful and stimulating department. His door was always open to those seeking advice and counsel. It is not surprising that he achieved so much during his relatively short trip to India last year while acting as project co-ordinator for a UNDP/UNESCO programme 'Special Assistance to Selected Science Departments in Indian Universities.' The United Nations could not have picked a better man for such a stamina-sapping mission.

Willie's tough professionalism really knew no boundaries; it overspilled onto many other areas including the sports field. His light blue tracksuit is a legend at Stirling University. Even beyond the campus his zest and vigour were manifest. At various times he was chairman of the local gardening club, a member of the male voice choir, and a coach to one of the Cub football teams. Just as Willie knew how to advise and encourage young academics, so too did he help the nine-year-old footballer. From his own student days as a keen footballer he remembered the importance of a new strip and the morale-boosting sing-song in the car returning from a 26-nil defeat.

All his many friends, both in the UK and abroad, have shared in one unique privilege—that of knowing the truly remarkable character of Willie Parker. For all of us, we are the beneficiaries of that enriching and rewarding experience, the memory of which will live on to compensate in part for our tragic loss which we share with his wife and three children.

James S. Roberts